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(71) Applicant: WYETH [US/US]; Five Giralda Farms, Madison, NJ 07940 (US).

(72) Inventors: TREPICCHIO, William, L.; 21 Abbot Bridge Drive, Andover, MA 01810 (US). OESTREICHER, Judith, L.; 125 Main Street, No. 62, Newmarket, NH 03857 (US). LEONARD, John, P.; 89 Tennyson Drive, Manchester, NH 03104 (US). DORNER, Andrew, J.; 20 Baskin Road, Lexington, MA 02173 (US). HUNTER, Sharon, E.; 28 9th Avenue, Haverhill, MA 01830 (US).

(74) Agents: MANDRA, Raymond, R. et al.; Fitzpatrick, Cella, Harper & Scinto, 30 Rockefeller Plaza, New York, NY 10112-3801 (US).

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(54) Title: METHODS FOR DIAGNOSING AND TREATING MULTIPLE SCLEROSIS AND COMPOSITIONS THEREOF

(57) Abstract: The present invention is directed to novel methods for diagnosis and prognosis of Multiple Sclerosis by identifying differentially expressed genes. Moreover, the present invention is also directed to methods that can be used to screen test compounds and therapies for the ability to inhibit multiple sclerosis. Additionally, methods and molecule targets (genes and their products) for therapeutic intervention in multiple sclerosis are described.

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TITLE

**METHODS FOR DIAGNOSING AND TREATING
MULTIPLE SCLEROSIS AND COMPOSITIONS THEREOF**

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[0001] This application claims benefit of U.S.
Provisional Patent Application No. 60/280,572, filed
March 30, 2001.

10 Field of the Invention

[0002] The present invention is directed to novel
methods for diagnosis and prognosis of Multiple
Sclerosis by identifying differentially expressed
15 genes. Additionally, methods and molecular targets
(genes and their products) for therapeutic intervention
in multiple sclerosis are described.

Background of the Invention

20

[0003] Multiple Sclerosis (MS) is a chronic, often
disabling autoimmune disease of the central nervous
system that first appears in young adults, with greater
occurrence in women than men. MS involves an
25 inflammation of the central nervous system, in which
lymphocytes attack myelin and oligodendrocytes to leave
behind characteristic lesions or plaques in the brain
and spinal cord. The involvement of complex

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immunological pathways, in particular systemic T-cell mediated pathways, has presented a challenge to researchers attempting to elucidate the mechanism of the disease.

5

[0003] Population studies on MS have suggested that MS is a product of genetic susceptibility as compounded by environmental exposure of an unknown nature. Genetic susceptibility of the disease has been borne out by a
10 correlated familial risk, showing the greatest recurrence among monozygotic twins, then among full-siblings and then cousins. Dyment et al. "Genetics of Multiple Sclerosis" Human Molecular Genetics, 6: 1693-1698 (1997). A connection to the environment has
15 further been drawn by several-fold differences in risk between populations in different latitudes, with increasing recurrence in higher latitudes with cooler climates (i.e. northern Europe or Canada). Dyment et al. Population studies however, have been unable to
20 clearly identify all of the genes involved in MS and their complex interaction, nor have these studies been able to provide significant guidance on treatment or therapy for MS.

25 [0004] The etiology of MS remains unclear, although numerous T-helper cell inflammatory pathways have been implicated in MS pathology. Due to the complex interaction of many genes expressed in these cell-mediated immune pathways, current methodologies have
30 yielded limited success in elucidating the disease. Various methods such as differential display, in situ hybridization, reverse transcriptase-polymerase chain reaction or competitive polymerase chain reaction have been used to study the patterns of gene expression with
35 limited success. Baranzini et al. "Transcriptional

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Analysis of Multiple Sclerosis Brain Lesions Reveals a Complex Pattern of Cytokine Expression," *J. Immunol.* 165: 6576-6582 (2000); van Boxel-Dezaire et al. "Decreased Interleukin-10 and Increased Interleukin-12p40 mRNA are Associated with Disease Activity and Characterize Different Disease Stages in Multiple Sclerosis" *Annals of Neurology*, 45: 695-703 (1999)). A study by Whitney and colleagues utilized genetic expression data from one patient to compare MS tissue to non-MS tissue, revealing that about sixty-two genes were differentially expressed, including the Duffy chemokine receptor, interferon regulatory factor-2, and tumor necrosis factor alpha receptor-2. Nonetheless, no method has yet been developed to provide comprehensive data on differentially expressed genes from multiple subjects at multiple stages of development, or through a wide range of geographical regions.

20 [0005] Furthermore, the nature and variability of MS as expressed in different individuals has proven to be a challenge in characterizing the disease and in providing a prognosis for each patient. Three general stages or types of MS have been characterized: (1) secondary progressive, in which symptoms and disability gradually worsen over time as opposed to having discrete recognizable attacks or relapses; (2) primary progressive, in which there are no attacks but a gradual worsening from the start; and (3) relapsing-remitting, in which there is a clinical worsening of symptoms followed temporary improvement. The present invention therefore addresses these issues by using differentially expressed genes to provide methods for diagnosis and prognosis, and assays for therapeutic intervention.

SUMMARY OF THE INVENTION

[0006] In one embodiment, the invention provides a
5 method of diagnosing a subject with multiple sclerosis
by comparing the level of expression of a marker in a
sample from a subject, where the marker is selected
from the group of markers set forth in Tables 1-5 (for
the animal model of multiple sclerosis) or 7-10, to the
10 normal level of expression of the marker in a control
sample, where a substantial difference between the
level of expression of the marker in the sample from
the subject and the normal level is an indication that
the subject is afflicted with multiple sclerosis. In a
15 preferred embodiment, the marker corresponds to a
transcribed polynucleotide or a portion thereof.
Preferably, the marker corresponds to a transcribed
polynucleotide or a portion thereof, and the sample is
collected from brain tissue or comprises peripheral
20 blood mononuclear cells (PBMCs). In another preferred
embodiment, the control sample is from non-involved
tissue from the subject. Alternatively, the control
sample is from the tissue of a nondiseased subject. In
a further preferred embodiment, the level of expression
25 of the marker in the sample differs from the normal
level of expression of the marker in a subject not
afflicted by a factor of at least two, and in an even
more preferred embodiment, the expression levels differ
by a factor of at least five.

30

[0007] In another preferred embodiment, the level of
expression of the marker in the sample is assessed by
detecting the presence in the sample of a protein
corresponding to the marker. In a particularly
35 preferred embodiment, the presence of the protein is

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detected using a reagent which specifically binds with the protein. In an even more preferred embodiment, the reagent comprises an antibody or fragments thereof. In another preferred embodiment, the method comprises a
5 marker selected from markers listed in Table 9 or 10. In another preferred embodiment, the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or portion thereof, where the
10 transcribed polynucleotide includes the marker. In a particularly preferred embodiment, the transcribed polynucleotide is an mRNA or a cDNA.

[0008] In yet another preferred embodiment, the level
15 of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or a portion thereof which hybridizes with a labeled probe under stringent conditions, wherein the transcribed polynucleotide comprises the marker

20

[0009] In another preferred embodiment for diagnosing a subject with multiple sclerosis, the level of expression in the sample of each of a panel of markers independently selected from the markers listed in
25 Tables 1-5 or 7-10 is compared with the normal level of expression of the same panel of markers in a control sample, where the level of expression of more than one of the markers is substantially different, relative to the corresponding normal levels of expression of the
30 markers, indicating that the subject is afflicted with multiple sclerosis. In a particularly preferred embodiment, the plurality includes at least five of the markers set forth in Tables 1-5 or 7-10.

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[0010] In another embodiment, the invention provides a method of monitoring the progression of multiple sclerosis in a subject, including detecting in a subject sample at a first point in time the expression
5 of marker, where the marker is selected from the group including the markers listed in Tables 1-5 or 7-10, repeating this detection step at a subsequent point in time with the same marker, and detecting a substantial difference between the levels of expression, thus
10 indicating that the subject has progressed to a different stage of multiple sclerosis. In a preferred embodiment, at least 5 markers are selected from the group of markers Tables 1-5 (murine) or 7-10 (human) and combinations thereof. In another preferred
15 embodiment, the marker corresponds to a transcribed polynucleotide or portion thereof, where the polynucleotide includes the marker. In a particularly preferred embodiment, the cells are collected from brain or blood tissue (PBMCs).

20

[0011] In another embodiment, the invention provides a method of assessing the efficacy of a test compound for inhibiting multiple sclerosis in a subject, including comparing expression of a marker in a first sample
25 obtained from the subject which is exposed to or maintained in the presence of the test compound, where the marker is selected from the group including the markers listed in Tables 1-5 (murine) or 7-10 (human), to expression of the marker in a second sample obtained
30 from the subject, where the second sample is not exposed to the test compound, where a substantially different level of expression of the marker in the first sample relative to that in the second sample is an indication that the test compound is efficacious for
35 inhibiting multiple sclerosis in the subject. In a

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preferred embodiment, the first and second samples are portions of a single sample obtained from the subject. In a particularly preferred embodiment, the substantially different level of expression is a lower
5 level of expression in the first sample.

[0012] In another embodiment, the invention provides a method of assessing the efficacy of a therapy for inhibiting multiple sclerosis in a subject, the method
10 including comparing expression of a marker in the first sample obtained from the subject prior to providing at least a portion of the therapy to the subject, where the marker is selected from the group including the markers listed in Tables 1-5 (murine) or 7-10 (human),
15 to expression of the marker in a second sample obtained from the subject following provision of the portion of the therapy, where a substantially different level of expression of the marker in the second sample relative to the first sample, is an indication that the therapy
20 is efficacious for inhibiting multiple sclerosis in the subject. In a preferred embodiment, the substantially different level of expression is a substantially lower level of expression in the second sample. In a particularly preferred embodiment, the method further
25 comprises a step of comparing expression of the marker in a control sample, where a substantially similar level of expression in the second sample, relative to the control sample, is an additional indication that the test compound is efficacious for inhibiting
30 multiple sclerosis.

[0013] In another embodiment, the invention provides a method of screening test compounds for inhibitors of multiple sclerosis in a subject, the method including
35 obtaining a sample including cells from a subject,

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separately maintaining aliquots of the sample in the presence of a plurality of test compounds, comparing expression of a marker in each of the aliquots, where the marker is selected from the group including the
5 markers listed in Tables 1-5 (murine) or 7-10 (human), and selecting one of the test compounds which induces a substantially different level of expression of the marker in the aliquot containing that test compound, relative to other test compounds. In a particularly
10 preferred embodiment, the substantially different level of expression is a substantially lower level of expression. In an alternative preferred embodiment, the substantially different level of expression is a substantially enhanced level of expression.

15

[0014] In another embodiment, the invention provides a kit for diagnosing a subject with multiple sclerosis, including reagents for assessing expression of a marker selected from the group including the markers listed in
20 Tables 1-5 (murine) or 7-10 (human).

[0015] In another embodiment, the invention provides a kit for diagnosing multiple sclerosis in a subject, the kit including a nucleic acid probe where the probe
25 specifically binds with a transcribed polynucleotide corresponding to a marker selected from the group including the markers listed in Tables 1-5 (murine) or 7-10 (human).

30 [0016] In another embodiment, the invention provides a kit for assessing the suitability of each of a plurality of compounds for inhibiting multiple sclerosis, the kit including a plurality of compounds and a reagent for assessing expression of a marker

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selected from the group including the markers listed in Tables 1-5 (murine) or 7-10 (human).

[0017] In another embodiment, the invention provides a
5 kit for diagnosing a subject with multiple sclerosis,
the kit including an antibody which specifically binds
with a protein corresponding to a marker selected from
the group including the markers listed in Tables 1-5
(murine) or 7-10 (human).

10

[0018] In another embodiment, the invention provides a
method of modulating the level of expression of a
marker selected from the markers listed in Tables 1-5
(murine) or 7-10 (human), the method comprising
15 providing to diseased cells of the subject an antisense
oligonucleotide complementary to a polynucleotide
corresponding to the marker.

[0019] In yet another embodiment, the invention
20 provides a method of modulating the level of expression
of a marker selected from the markers listed in Tables
1-5 (murine) or 7-10 (human), the method comprising
providing to diseased cells of a subject a protein. In
a particularly preferred embodiment, the invention
25 further provides a vector which comprises a
polynucleotide encoding the protein.

[0020] In another embodiment, the invention provides a
method of modulating a level of expression of a marker
30 selected from the markers listed in Tables 1-5 (murine)
or 7-10 (human), where the method comprises providing
to diseased cells of a subject an antibody. In a
particularly preferred embodiment, the method further
comprises a therapeutic moiety conjugated to the
35 antibody.

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[0021] In another preferred embodiment, the invention provides a method of localizing a therapeutic moiety to diseased tissue of a subject comprising exposing the tissue to an antibody which is specific to a protein
5 encoded by a marker listed in Tables 1-5 (murine) or 7-10 (human).

[0022] In another preferred embodiment, the present invention provides a method of screening for a test
10 compound capable of modulating the activity of a protein encoded from a marker listed in Tables 1-5 (murine) or 7-10 (human), said method comprising combining said protein and test compound, and determining the effect of said test compound on the
15 therapeutic efficacy of said protein.

[0023] In yet another preferred embodiment, the present invention provides a method of screening for a bioactive agent capable of interfering with the binding
20 of a protein or a fragment thereof and an antibody which binds to said protein or fragment thereof, where the method combines a protein or fragment thereof, a bioactive agent and an antibody which binds to the protein or fragment thereof, wherein the method further
25 includes determining the binding of the protein or fragment thereof and the antibody.

[0024] In another preferred embodiment, the present invention provides an antibody which specifically binds
30 to a protein encoded from a marker listed in Tables 1-5 (murine) or 7-10 (human). In particularly preferred embodiment, the antibody is monoclonal and humanized.

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[0026] In yet another preferred embodiment, the present invention provides a peptide encoded from markers listed in Tables 1-5 (murine) or 7-10 (human). Furthermore, the present invention is also directed to
5 a composition comprising the peptide.

[0027] In an alternative embodiment, the present invention provides a composition capable of modulating an immune response in a subject, where the composition
10 comprises a protein encoded from a marker listed in Tables 1-5 (murine) or 7-10 (human) and a pharmaceutically acceptable carrier.

[0028] In yet another embodiment, the present
15 invention provides a biochip comprising a panel of markers selected from the group of markers listed in Tables 1-5 (murine) or 7-10 (human). Furthermore, in a particularly preferred embodiment, the markers for a biochip may be selected for subjects suspected of
20 having multiple sclerosis from different stages of the disease: secondary progressive, primary progressive, relapsing-remitting. In a still another embodiment, the markers may be selected for a subject which is from a higher-risk geographical region.

25

[0029] Other features and advantages of the invention will be apparent from the following detailed description and claims.

30

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIGURE 1A through 1D depict the relative expression levels of four cytokines, IL-10 (FIGURE 1A; $p < 0.4$), IL-8 (FIGURE 1B; $p < 0.027$), IL-12p35 (FIGURE 1C;

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p<0.028) and IL-1 β (FIGURE 1D; p<0.011), in MS-afflicted subjects versus nondiseased subjects, as measured in Example 2(D) below. The sample size was 10 MS-afflicted subjects and 10 nondiseased subjects.

5

[0031] FIGURE 2 depicts the results of the class predictor used in Example 2(E), wherein a diagnosis of "normal" is indicated as positive and "MS-afflicted" are indicated as negative. The model was based on
10 markers listed in Table 10, as expressed in human blood samples.

DETAILED DESCRIPTION OF THE INVENTION

[0032] The present invention provides methods for
15 diagnosis and prognosis evaluation for multiple sclerosis (MS) in subjects, as well as methods and molecular targets for therapeutic intervention.

[0033] In one aspect of the invention, the expression
20 levels of genes are determined in a particular patient sample for which either diagnosis or prognosis information is desired. The level of expression of a number of genes simultaneously provides an expression profile, which is essentially a "fingerprint" of the
25 activity of a gene or plurality of genes that is unique to the state of the cell. Comparison of relative levels of expression have been found to be indicative of the presence of multiple sclerosis, and as such permits for diagnostic and prognostic analysis. By
30 comparing relative expression profiles of multiple sclerosis tissue in known different states (i.e. secondary progressive vs. primary progressive vs. relapsing-remitting), information regarding which genes are important (including both up- and down-regulation
35 of genes) in each of these states is obtained. The

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identification of gene markers that are differentially expressed in diseased versus non-diseased tissue, as well as differential expression resulting in different prognostic outcomes, allows the use of this invention in a number of ways. For example, the evaluation of a particular treatment regime may be evaluated: will a particular drug act to improve the long-term prognosis in a particular patient? The discovery of these differential expression patterns for individual genes allows for screening of drug candidates with an eye to mimicking or altering a particular expression pattern; for example, screening can be done for drugs that will alter the MS differential expression pattern or convert a poor prognosis pattern to a better prognosis pattern. This may be done by making biochips comprising sets of the significant MS genes, which can then be used in these screens. These methods can also be done on the protein basis; that is protein expression levels of the MS-associated proteins can be evaluated for diagnostic and prognostic purposes or to screen test compounds. In addition, the markers can be administered for gene therapy purposes, including the administration of antisense nucleic acids, or proteins (including antibodies and other modulators thereof) administered as therapeutic drugs.

[0034] Moreover, in a preferred embodiment, the relative expression levels are measured from human samples; however, as will be appreciated by those in the art, expression levels of markers from other organisms may be useful in animal models of disease and drug evaluation; thus other markers are provided, from vertebrates, including mammals, including rodents (mice, hamsters, rats, guinea pigs, etc), primates, farm animals (including sheep, goats, pigs, cows,

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horses, etc). Markers from other organisms may be obtained using the techniques outlined below.

[0035] The present invention is based, at least in part, on the identification of a number of genetic markers, set forth in Tables 1-5 (mouse) and 7-10 (human), which are differentially expressed between diseased samples (MS-associated) and non-diseased samples. Using a murine model of experimental autoimmune encephalitis ("EAE") as an analogy to human MS, a panel of 11,000 known murine genes was screened for expression in diseased versus non-diseased tissue from twelve different mice afflicted with the disease (see Example 1). Those genes with statistically substantial differences between the diseased and normal tissues are identified in Tables 1-2. This differential expression was observed either as an increase in expression (Table 1), or a decrease in expression (Table 2). In addition, to narrow the subset of diseased-related, immune-mediated genes, diseased cells were stimulated *in vitro* with a growth protein and with an inhibitory compound, to yield 6 genes which were differentially expressed: CAPN12 (calpain 12), MT1 (metallothionein 1), MYO1F (myosin 11), TLN (talin), UNK_AA117532 (EST), and UNK_AA645990 (EST).

[0036] Using the murine model as a springboard, a panel of 12,000 human genes was likewise screened for differential expression between diseased samples of the brain and blood versus non-diseased samples (either from noninvolved brain tissue or from non-diseased subjects) (see Example 2). Genes that were differentially up- or down- regulated in MS blood (peripheral blood mononuclear cells, or "PBMCs")

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samples are shown in Table 7, while genes that were differentially up- or down-regulated in MS brain samples are shown in Table 8. Furthermore, comparison of the differentially expressed genes between blood and
5 brain samples yielded 181 genes in common. Further comparison of the 181 genes to the murine genes that were differentially regulated *in vitro* yielded six genes (shown in Table 9 with their accession numbers): BEEF1D, PIM2, PRDX2, SEC24C, UNK_AJ24 AND XIP.

10

[0037] Included among the genes used to screen diseased versus non-diseased tissue in the murine panel were two genes known in the art to be implicated in EAE: TNF- α and IFN- β . These genes served as an internal control.
15 Each of these genes were found to be substantially increased in expression in EAE cells as opposed to non-diseased cells, thus validating the method as a means for identifying significant genes involved in EAE pathology. Correspondingly, the genes which are known
20 in the art to be linked to MS (listed in Table 6) may also serve as validation in expression studies for MS. Moreover, the differentially regulated genes of the invention, as listed in Tables 1-5 (murine) or 7-10 (human), have not been previously associated with EAE
25 or multiple sclerosis.

[0038] Accordingly, the present invention pertains to the use of the genes set forth in Tables 1-5 (murine) or 7-10 (human), the corresponding mRNA transcripts,
30 and the encoded polypeptides as markers for the presence or risk of development of MS. These markers are further useful to correlate the extent and/or severity of disease. In particular, the present invention is directed to the genes set forth in Table 9
35 (genes which were shown to be differentially regulated

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in both murine and human models of MS), and in Table 10, the genes which were used in the MS class predictor of Example 2(E). Panels of the markers can be conveniently arrayed on solid supports, i.e. biochips
5 for use in kits. Markers can also be useful for assessing the efficacy of a treatment or therapy of MS.

[0039] In one aspect, the invention provides markers whose level of expression, which signifies their
10 quantity or activity, is correlated with the presence of MS. The markers of the invention may be nucleic acid molecules (e.g., DNA, cDNA or mRNA) or peptide(s). Preferably the invention is performed by detecting the presence of a transcribed polynucleotide or a portion
15 thereof, wherein the transcribed polynucleotide comprises the marker. Alternatively, detection may be performed by detecting the presence of a protein which corresponds to the marker. The markers of the invention are either increased or decreased in quantity
20 or activity in MS tissue as compared to non-diseased tissue. For example, the gene designated 'SAA3' is increased in expression level in murine EAE cells, relative to control cells, while the gene designated 'E_TC36651_s' is decreased in expression level in
25 murine EAE cells, relative to control cells. Both the presence of increased or decreased mRNA for these genes (and for other genes set forth in Tables 1-5 and 7-10), and also increased or decreased levels of the protein products of these genes (and other genes set forth in
30 Tables 1-5 and 7-10) serve as markers for either EAE or MS. Preferably, increased or decreased levels of the markers of the invention are increases and decreases of a magnitude that are statistically substantial as compared to appropriate control samples (i.e., non-

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involved tissue or from non-diseased subjects.) In particularly preferred embodiments, the marker is increased or decreased relative to control samples by at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10- fold or more. Similarly one skilled in the art will be cognizant of the fact that a preferred detection methodology is one in which the resulting detection values are above the minimum detection limit of the methodology.

10

[0040] Detection and measurement of the relative amount of a nucleic acid or peptide marker of the invention may be by any method known in the art (see, i.e., Sambrook, J., Fritsh, E.F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and *Current Protocols in Molecular Biology*, eds. Ausubel et al, John Wiley & Sons (1992)). Typical methodologies for detection of a transcribed polynucleotide include RNA extraction from a cell or tissue sample, followed by hybridization of a labeled probe (i.e., a complementary nucleic acid molecule) specific for the target RNA to the extracted RNA and detection of the probe (i.e. Northern blotting). Typical methodologies for peptide detection include protein extraction from a cell or tissue sample, followed by hybridization of a labeled probe (i.e., an antibody) specific for the target protein to the protein sample, and detection of the probe. The label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Detection of specific peptide(s) and nucleic acid molecules may also be assessed by gel electrophoresis, column chromatography, direct sequencing, or quantitative PCR (in the case of nucleic

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acid molecules) among many other techniques well known to those skilled in the art.

[0041] In certain embodiments, the genes themselves
5 (i.e., the DNA or cDNA) may serve as markers for MS.
For example, the absence of nucleic acids corresponding to a gene (i.e. a gene from Table 2) such as by deletion of all or part of the gene, may be correlated with disease. Similarly an increase of nucleic acid
10 corresponding to a gene (i.e. a gene from Tables 1-5 and 7-10), such as by duplication of the gene, may also be correlated with disease.

[0042] Detection of the presence or number of copies
15 of all or a part of a marker gene of the invention may be performed using any method known in the art.
Typically, it is convenient to assess the presence and/or quantity of a DNA or cDNA by Southern analysis, in which total DNA from a cell or tissue sample is
20 extracted, is hybridized with a labeled probe (i.e. a complementary DNA molecules), and the probe is detected. The label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Other useful methods of DNA detection and/or
25 quantification include direct sequencing, gel electrophoresis, column chromatography, and quantitative PCR, as is known by one skilled in the art.

30 [0043] The invention also encompasses nucleic acid and peptide molecules which are structurally different from the molecules described above (i.e. which have a slight altered nucleic acid or amino acid sequence), but which have the same properties as the molecules above (e.g.,
35 encoded amino acid sequences, or which are changed only

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in nonessential amino acid residues). Such molecules include allelic variants, and are described in greater detail in subsection I.

5 [0044] In another aspect, the invention provides markers whose quantity or activity is correlated with different stages of MS: secondary progressive, primary progressive, relapsing-remitting. These markers are either increased or decreased in quantity or activity
10 in MS tissue in a fashion that is either positively or negatively correlated with the degree of severity of the MS. A method of monitoring progression of MS in subjects may be devised by detecting a substantial difference between the levels of expression in a
15 diseased subject at different points in time. The subsequent level of expression may further be compared to different expression profiles of various MS stages to confirm whether the subject has a matching profile. In yet another aspect, the invention provides markers
20 whose quantity or activity is correlated with a risk in a subject for developing MS. For instance, the markers may be selected for higher-risk geographical regions. These markers are either increased or decreased in activity or quantity in direct correlation to the
25 likelihood of the development of MS in a subject.

[0045] Each marker may be considered individually, although it is within the scope of the invention to provide combinations of two or more markers for use in
30 the methods and compositions of the invention to increase the confidence of the analysis. In another aspect, the invention provides panels of the markers of the invention. In a preferred embodiment, these panels of markers are selected such that the markers within
35 any one panel share certain features (see Example

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2(E)). For example, the markers of a first panel may each exhibit a decrease in quantity or activity in MS tissue as compared to samples from non-involved samples from the same subject or tissue from a non-diseased
5 subject. Similarly, different panels of markers may be composed of markers from different tissues (i.e., blood (Table 7) or brain tissue (Table 8)), or may represent different components of an MS stage or type (i.e.,
10 secondary progressive, primary progressive, relapsing-remitting in MS humans; or onset, peak and recovery in EAE mice). Panels of the markers of the invention may be made by independently selecting markers from any of Tables 1-5 for mice, and Tables 7-10 for humans, and may further be provided on biochips, as discussed
15 below.

[0046] It will be appreciated by one skilled in the art that the panels of markers of the invention may conveniently be provided on solid supports, as a
20 biochip. For example, polynucleotides may be coupled to an array (e.g., a biochip using GeneChip® for hybridization analysis), to a resin (e.g., a resin which can be packed into a column for column
25 chromatography), or a matrix (e.g. a nitrocellulose matrix for northern blot analysis). The immobilization of molecules complementary to the marker(s), either covalently or noncovalently, permits a discrete analysis of the presence or activity of each marker in a sample. In an array, for example, polynucleotides
30 complementary to each member of a panel of markers may individually be attached to different, known locations on the array. The array may be hybridized with, for example, polynucleotides extracted from a brain sample from a subject. The hybridization of polynucleotides
35 from the sample with the array at any location on the

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array can be detected, and thus the presence or quantity of the marker in the sample can be ascertained. In a preferred embodiment, an array based on a biochip is employed. Similarly, Western analyses
5 may be performed on immobilized antibodies specific for different polypeptide markers hybridized to a protein sample from a subject.

[0047] It will also be apparent to one skilled in the
10 art that the entire marker protein or nucleic acid molecule need not be conjugated to the biochip support; a portion of the marker or sufficient length for detection purposes (i.e., for hybridization), for example a portion of the marker which is 7, 10, 15, 20,
15 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 100 or more nucleotides or amino acids in length may be sufficient for detection purposes.

[0048] The nucleic acid and peptide markers of the
20 invention may be isolated from any tissue or cell of a subject. In a preferred embodiment, the tissue is brain tissue. However, it will be apparent to one skilled in the art that other tissue samples, including bodily fluids such as blood, may also serve as sources
25 from which the markers of the invention may be assessed. The tissue samples containing one or more of the markers themselves may be useful in the methods of the invention, and one skilled in the art will be cognizant of the methods by which such samples may be
30 conveniently obtained, stored and/or preserved.

[0049] Several markers were known prior to the invention to be associated with MS and are provided in Table 6. These markers are not to be considered as

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markers of the invention. However, these markers may be conveniently be used in combination with the markers of the invention (Tables 1-5 and 7-10) in the methods, panels and kits of the invention.

5

[0050] In another aspect, the invention provides methods of making an isolated hybridoma which produces an antibody useful for diagnosing a patient with MS. In this method, a protein corresponding to a marker of the invention is isolated (e.g., by purification from a cell in which it is expressed or by transcription and translation of a nucleic acid encoding the protein *in vivo* or *in vitro* using known methods). A vertebrate, preferably a mammal such as a mouse, rabbit or sheep, is immunized using the isolated protein or protein fragment. The vertebrate may optionally (and preferably) be immunized at least one additional time with the isolated protein or protein fragment, so that the vertebrate exhibits a robust immune response to the protein or protein fragment. Splenocytes are isolated from the immunized vertebrate and fused with an immortalized cell line to form hybridomas, using any of a variety of methods well known in the art. Hybridomas formed in this manner are then screened using standard methods to identify one or more hybridomas which produce an antibody which specifically binds with the protein or protein fragment. The invention also includes hybridomas made by this method and antibodies made using such hybridomas.

25
30

[0051] The invention provides methods of diagnosing MS, or determining the risk of developing MS. These methods involve isolating a sample from a subject (e.g., a sample containing blood cells or brain cells), detecting the presence, quantity and/or activity of one

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or more markers of the invention in the sample relative to a second sample from a non-diseased subject, or from a non-involved tissue in the same subject. The levels of markers in the two samples are compared, and a
5 substantial increase or decrease in one or more markers in the test sample indicates the presence or risk of presence of MS in the subject.

[0052] The invention also provides methods of
10 assessing the efficacy of a test compound or therapy for inhibiting MS in a subject. These methods involve isolating samples from a subject suffering from MS who is undergoing treatment or therapy, and detecting the presence, quantity, and/or activity of one or more
15 markers of the invention in the first sample relative to a second sample. Where a test compound is administered, the first and second samples are preferably sub-portions of a single sample taken from the patient, wherein the first portion is exposed to
20 the test compound and the second portion is not. In one aspect of this embodiment, the substantially different level of expression is a substantially lower level of expression in the first sample, relative to the second. Most preferably, the level of expression
25 in the first sample approximates (i.e., less than a two fold difference from a control) the level of expression in a third control sample, taken from either a non-diseased subject or non-involved tissue.

30 [0053] Where the efficacy of a therapy is being assessed, the first sample obtained from the subject is preferably obtained prior to provision of at least a portion of the therapy, whereas the second sample is obtained following provision of the portion of the
35 therapy. The levels of markers in the samples are

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compared, preferably against a third control sample as well, and correlated with the presence, risk of presence, or severity of MS. Most preferably, the level of markers in the second sample approximates the
5 level of expression of a third control sample. By assessing whether expression of MS has been lessened or alleviated in the sample, the ability of the treatment or therapy to treat MS is determined.

10 [0054] The invention also provides a method of screening test compounds for inhibitors of MS, and to the pharmaceutical compositions comprising the test compounds. The method of screening comprises obtaining samples of diseased or involved cells, maintaining
15 separate aliquots of the samples with a plurality of test compounds, and comparing expression of a marker in each of the aliquots to determine whether any of the test compounds provides a substantially different level of expression from a control. In addition, methods of
20 screening may be devised by combining a test compound with a protein and thereby determining the effect of the test compound on the protein. Alternatively, the invention is further directed to a method of screening for bioactive agents capable of interfering with the
25 binding of a protein encoded by the markers of Tables 1-5 (murine) or 7-10 (human), and an antibody, by combining the bioactive agent, protein, and antibody together and determining whether binding of the antibody and protein occurs.

30

[0055] Moreover, the invention is directed to pharmaceutical compositions comprising the test compound, or bioactive agent, which may further include a marker protein and/or nucleic acid of the invention
35 (e.g., for those markers in Tables 1-5 or 7-10 which

- 25 -

are decreased or increased in quantity or activity in MS versus non-diseased tissue), and can be formulated as described herein. Alternatively, these compositions may include an antibody which specifically binds to a marker protein of the invention and/or an antisense nucleic acid molecule which is complementary to a marker nucleic acid of the invention (e.g., for those markers which are increased in quantity in MS tissue) and can be formulated as described herein.

10

[0056] The invention further provides methods of modulating a level of expression of a marker of the invention, comprising administration to the diseased cells of the subject a variety of compositions which correspond to the markers of Tables 1-5 (murine) or 7-10 (human), including proteins or antisense oligonucleotides. The protein may be provided to the diseased cells by further providing a vector comprising a polynucleotide encoding the protein to the cells.

Alternatively, the expression levels of the markers of the invention may be modulated by providing an antibody, a plurality of antibodies or an antibody conjugated to a therapeutic moiety. Treatment with the antibody may further be localized to the diseased tissue. In another aspect, the invention provides methods for localizing a therapeutic moiety to diseased tissue comprising exposing the tissue to an antibody which is specific to a protein encoded from the markers of the invention. This method may therefore provide a means to inhibit or enhance expression of a specific gene corresponding to a marker listed in Tables 1-5 or 7-10. Where the gene is up-regulated as a result of MS pathology, it is likely that inhibition of MS progression would involve inhibiting expression of the up-regulated gene. As a corollary to this method,

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where the gene is down-regulated, inhibition of MS progression would therefore likely require enhancing expression of the down-regulated gene.

5 [0057] In another aspect, the invention includes antibodies that are specific to proteins corresponding to markers of the invention. Preferably the antibodies are monoclonal, and most preferably, the antibodies are humanized, as per the description of antibodies
10 described below.

[0058] In still another aspect of the invention, the invention includes peptides or proteins which are encoded from the markers of the invention, and to
15 compositions thereof.

[0059] The invention also provides kits for diagnosing a subject with MS, the kit comprising reagents for assessing expression of the markers of the invention.
20 Preferably, the reagents may be an antibody or fragment thereof, wherein the antibody or fragment thereof specifically binds with a protein corresponding to a marker from Tables 1-5 or 7-10. Optionally, the kits may comprise a nucleic acid probe wherein the probe
25 specifically binds with a transcribed polynucleotide corresponding to a marker selected from the group consisting of the markers listed in Tables 1-5 or 7-10.

30 [0060] The invention further provides kits for assessing the suitability of each of a plurality of compounds for inhibiting progression of MS in a subject. Such kits include a plurality of compounds to be tested, and a reagent (i.e. antibody specific to

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corresponding proteins of the invention) for assessing expression of a marker listed in Tables 1-5 or 7-10.

[0061] Modifications to the above-described

5 compositions and methods of the invention, according to standard techniques, will be readily apparent to one skilled in the art and are meant to be encompassed by the invention.

10 [0062] To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

[0063] As used herein, the term "modulation" includes,
15 in its various grammatical forms (e.g., "modulated", "modulation", "modulating", etc.), up-regulation, induction, stimulation, potentiation, and/or relief of inhibition, as well as inhibition and/or down-regulation.

20

[0064] As used herein, the terms "polynucleotide" and "oligonucleotide" are used interchangeably, and include polymeric forms of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs
25 thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, messenger RNA (mRNA), transfer RNA,
30 ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such

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as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The term also includes both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[0065] A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for guanine when the polynucleotide is RNA. This, the term "polynucleotide sequence" is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be inputted into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching.

25

[0066] A "gene" includes a polynucleotide containing at least one open reading frame that is capable of encoding a particular polypeptide or protein after being transcribed and translated. Any of the polynucleotide sequences described herein may be used to identify larger fragments or full-length coding sequences of the gene with which they are associated. Methods of isolating larger fragment sequences are known to those of skill in the art, some of which are described herein.

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[0067] A "gene product" includes an amino acid sequence (e.g., peptide or polypeptide) generated when a gene is transcribed and translated.

5

[0068] As used herein, a "polynucleotide corresponds to" another (a first) polynucleotide if it is related to the first polynucleotide by any of the following relationships:

10

1) The second polynucleotide comprises the first polynucleotide and the second polynucleotide encodes a gene product.

15 2) The second polynucleotide is 5' or 3' to the first polynucleotide in cDNA, RNA, genomic DNA, or fragments of any of these polynucleotides. For example, a second polynucleotide may be a fragment of a gene that includes the first and second polynucleotides. The
20 first and second polynucleotides are related in that they are components of the gene coding for a gene product, such as a protein or antibody. However, it is not necessary that the second polynucleotide comprises or overlaps with the first polynucleotide to be
25 encompassed within the definition of "corresponding to" as used herein. For example, the first polynucleotide may be a fragment of a 3' untranslated region of the second polynucleotide. The first and second polynucleotide may be fragments of a gene coding for a
30 gene product. The second polynucleotide may be an exon of the gene while the first polynucleotide may be an intron of the gene.

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3) The second polynucleotide is the complement of the first polynucleotide.

[0069] As used herein, the term, "transcribed" or
5 "transcription" refers to the process by which genetic code information is transferred from one kind of nucleic acid to another, and refers in particular to the process by which a base sequence of mRNA is synthesized on a template of cDNA.

10

[0070] A "probe" when used in the context of polynucleotide manipulation includes an oligonucleotide that is provided as a reagent to detect a target present in a sample of interest by hybridizing with the
15 target. Usually, a probe will comprise a label or a means by which a label can be attached, either before or subsequent to the hybridization reaction. Suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and
20 proteins, including enzymes.

[0071] A "primer" includes a short polynucleotide, generally with a free 3'-OH group that binds to a target or "template" present in a sample of interest by
25 hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target. A "polymerase chain reaction" ("PCR") is a reaction in which replicate copies are made of a target polynucleotide using a "pair of primers" or "set or
30 primers" consisting of "upstream" and a "downstream" primer, and a catalyst of polymerization, such as a DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are well known in the art, and are taught, for example, in MacPherson et al., IRL
35 Press at Oxford University Press (1991)). All

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processes of producing replicate copies of a polynucleotide, such as PCR or gene cloning, are collectively referred to herein as "replication". A primer can also be used as a probe in hybridization reactions, such as Southern or Northern blot analyses (see, e.g., Sambrook, J., Fritsh, E.F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

10

[0072] The term "cDNAs" includes complementary DNA, that is mRNA molecules present in a cell or organism made into cDNA with an enzyme such as reverse transcriptase. A "cDNA library" includes a collection of mRNA molecules present in a cell or organism, converted into cDNA molecules with the enzyme reverse transcriptase, then inserted into "vectors" (other DNA molecules that can continue to replicate after addition of foreign DNA). Exemplary vectors for libraries include bacteriophage, viruses that infect bacteria (e.g., lambda phage). The library can then be probed for the specific cDNA (and thus mRNA) of interest.

[0073] A "gene delivery vehicle" includes a molecule that is capable of inserting one or more polynucleotides into a host cell. Examples of gene delivery vehicles are liposomes, biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria, viruses and viral vectors, such as baculovirus, adenovirus, and retrovirus, bacteriophage, cosmid, plasmid, fungal vector and other recombination vehicles typically used in the art which

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have been described for replication and/or expression in a variety of eukaryotic and prokaryotic hosts. The gene delivery vehicles may be used for replication of the inserted polynucleotide, gene therapy as well as
5 for simply polypeptide and protein expression.

[0074] A "vector" includes a self-replicating nucleic acid molecule that transfers an inserted polynucleotide into and/or between host cells. The term is intended
10 to include vectors that function primarily for insertion of a nucleic acid molecule into a cell, replication vectors that function primarily for the replication of nucleic acid and expression vectors that function for transcription and/or translation of the
15 DNA or RNA. Also intended are vectors that provide more than one of the above function.

[0075] A "host cell" is intended to include any individual cell or cell culture which can be or has
20 been a recipient for vectors or for the incorporation of exogenous nucleic acid molecules, polynucleotides and/or proteins. It also is intended to include progeny of a single cell. The progeny may not necessarily be completely identical (in morphology or
25 in genomic or total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. The cells may be prokaryotic or eukaryotic, and include but are not limited to bacterial cells, yeast cells, insect cells, animal cells, and mammalian
30 cells, e.g., murine, rat, simian or human cells.

[0076] The term "genetically modified" includes a cell containing and/or expressing a foreign gene or nucleic acid sequence which in turn modifies the genotype or

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phenotype of the cell or its progeny. This term includes any addition, deletion, or disruption to a cell's endogenous nucleotides.

- 5 [0077] As used herein, "expression" includes the process by which polynucleotides are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the
- 10 mRNA, if an appropriate eukaryotic host is selected. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector
- 15 includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold
- 20 Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment
- 25 of the ribosome. Such vectors can be obtained commercially or assembled by the sequences described in methods well known in the art, for example, the methods described below for constructing vectors in general.
- 30 [0078] "Differentially expressed", as applied to a gene, includes the differential production of mRNA transcribed from a gene or a protein product encoded by the gene. A differentially expressed gene may be overexpressed or underexpressed as compared to the
- 35 expression level of a normal or control cell. In one

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aspect, it includes a differential that is 2 times, preferably 5 times or preferably 10 times higher or lower than the expression level detected in a control sample. The term "differentially expressed" also
5 includes nucleotide sequences in a cell or tissue which are expressed where silent in a control cell or not expressed where expressed in a control cell.

[0079] The term "polypeptide" includes a compound of
10 two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc. As used herein the term "amino acid" includes either
15 natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly referred to as an oligopeptide. Peptide chains of greater than three
20 or more amino acids are referred to as a polypeptide or a protein.

[0080] "Hybridization" includes a reaction in which one or more polynucleotides react to form a complex
25 that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex
30 structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic
35 cleavage of a polynucleotide by a ribozyme.

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[0081] Hybridization reactions can be performed under conditions of different "stringency". The stringency of a hybridization reaction includes the difficulty with which any two nucleic acid molecules will hybridize to one another. The present invention also includes polynucleotides capable of hybridizing under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in Table A below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

Table A. Stringency Conditions

	Stringency Condition	Poly-nucleotide Hybrid	Hybrid Length (bp) ¹	Hybridization Temperature and Buffer ¹	Wash Temperature and Buffer ¹
5	A	DNA:DNA	> 50	65°C; 1xSSC -or- 42°C; 1xSSC, 50% formamide	65°C; 0.3xSSC
	B	DNA:DNA	<50	T _B *; 1xSSC	T _B *; 1xSSC
	C	DNA:RNA	> 50	67°C; 1xSSC -or- 45°C; 1xSSC, 50% formamide	67°C; 0.3xSSC
	D	DNA:RNA	<50	T _D *; 1xSSC	T _D *; 1xSSC
	E	RNA:RNA	>50	70°C; 1xSSC -or- 50°C; 1xSSC, 50% formamide	70°C; 0.3xSSC
10	F	RNA:RNA	<50	T _F *; 1xSSC	T _F *; 1xSSC
	G	DNA:DNA	> 50	65°C; 4xSSC -or- 42°C; 4xSSC, 50% formamide	65°C; 1xSSC
	H	DNA:DNA	<50	T _H *; 4xSSC	T _H *; 4xSSC
	I	DNA:RNA	> 50	67°C; 4xSSC -or- 45°C; 4xSSC, 50% formamide	67°C; 1xSSC
	J	DNA:RNA	<50	T _J *; 4xSSC	T _J *; 4xSSC
15	K	RNA:RNA	> 50	70°C; 4xSSC -or- 50°C; 4xSSC, 50% formamide	67°C; 1xSSC
	L	RNA:RNA	<50	T _L *; 2xSSC	T _L *; 2xSSC
	M	DNA:DNA	> 50	50°C; 4xSSC -or- 40°C; 6xSSC, 50% formamide	50°C; 2xSSC
	N	DNA:DNA	<50	T _N *; 6xSSC	T _N *; 6xSSC
	O	DNA:RNA	> 50	55°C; 4xSSC -or- 42°C; 6xSSC, 50% formamide	55°C; 2xSSC
20	P	DNA:RNA	<50	T _P *; 6xSSC	T _P *; 6xSSC
	Q	RNA:RNA	> 50	60°C; 4xSSC -or- 45°C; 6xSSC, 50% formamide	60°C; 2xSSC
	R	RNA:RNA	<50	T _R *; 4xSSC	T _R *; 4xSSC

1: The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

15 ¹: SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

35 T_B* - T_R*: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(°C) = 81.5 + 16.6(log₁₀Na⁺) + 0.41(%G+C) - (600/N), where N is the number of bases in the hybrid, and Na⁺ is the concentration of sodium ions in the hybridization buffer (Na⁺ for 1xSSC = 0.165 M).

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Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and Current Protocols in Molecular Biology, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

10

[0082] When hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides, the reaction is called "annealing" and those polynucleotides are described as "complementary".
15 A double-stranded polynucleotide can be "complementary" or "homologous" to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second.
"Complementarity" or "homology" (the degree that one
20 polynucleotide is complementary with another) is quantifiable in terms of the proportion of bases in opposing strands that are expected to hydrogen bond with each other, according to generally accepted base-pairing rules.

25

[0083] An "antibody" includes an immunoglobulin molecule capable of binding an epitope present on an antigen. As used herein, the term encompasses not only intact immunoglobulin molecules such as monoclonal and
30 polyclonal antibodies, but also anti-idotypic antibodies, mutants, fragments, fusion proteins, bi-specific antibodies, humanized proteins, and modifications of the immunoglobulin molecule that comprises an antigen recognition site of the required
35 specificity.

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[0084] As used herein, the term "diseased" refers to cells, tissues or samples from a subject afflicted with multiple sclerosis, wherein the cell, tissue or sample
5 has been affected by multiple sclerosis (i.e. from white-matter lesion). As used herein, the term "non-diseased" refers to cells, tissues or other such samples taken from a subject who is not afflicted with multiple sclerosis. As used herein, "non-involved"
10 refers to cells, tissues, or samples wherein the tissue is from a subjected afflicted with MS, but wherein the cells, tissues or samples are believed to be unaffected by multiple sclerosis. Preferred tissue (and cell) samples are from brain, blood, sera, lymph, thymus,
15 spleen, bone marrow or pus. Most preferred samples are peripheral blood mononuclear cells ("PBMC") or brain tissue.

[0085] As used herein, the term "marker" includes a polynucleotide or polypeptide molecule which is present
20 or absent, or increased or decreased in quantity or activity in subjects afflicted with multiple sclerosis, or in MS-associated cells. The relative change in quantity or activity of the marker is correlated with the incidence or risk of incidence of multiple
25 sclerosis.

[0086] As used herein, the term "panel of markers" includes a group of markers, the quantity or activity of each member of which is correlated with the
30 incidence or risk of incidence of a MS-associated condition. In certain embodiments, a panel of markers may include only those markers which are either increased or decreased in quantity or activity in subjects afflicted with or cells involved in a MS-
35 associated condition. In a preferred embodiment, the

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panel of markers comprises at least 5 markers, and most preferably, the panel comprises markers listed in Table 10. In other embodiments, a panel of markers may include only those markers present in a specific tissue type which are correlated with the incidence of risk of incidence of a MS-associated condition.

Various aspects of the invention are described in further detail in the following subsections:

10

I. Isolated Nucleic Acid Molecules

[0087] One aspect of the invention pertains to isolated nucleic acid molecules that either themselves are the genetic markers (e.g., mRNA) of the invention, or which encode the polypeptide markers of the invention, or fragments thereof. Another aspect of the invention pertains to isolated nucleic acid fragments sufficient for use as hybridization probes to identify the nucleic acid molecules encoding the markers for the invention in a sample, as well as nucleotide fragments for use as PCR primers of the amplification or mutation of the nucleic acid molecules which encode the markers of the invention. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA..

[0088] The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example,

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with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated marker nucleic acid molecule of the invention, or nucleic acid molecule encoding a polypeptide marker of the invention, can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

[0089] A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of one of the genes set forth in Tables 1-5 or 7-10, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of one of the genes set forth in Tables 1-5 or 7-10 as a hybridization probe, a marker gene of the invention or a nucleic acid molecule encoding a polypeptide marker of the invention can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis,

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T. Molecular Cloning: A Laboratory Manual 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold spring Harbor, NY, 1989).

5 [0090] A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be
10 cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to marker nucleotide sequences, or nucleotide sequences encoding a marker of the invention can be prepared by standard synthetic techniques, e.g.,
15 using an automated DNA synthesizer.

[0091] In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the
20 nucleotide sequence of a marker of the invention (e.g., a gene set forth in Tables 1-5 or 7-10), or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to such a nucleotide sequence is one which is sufficiently complementary to
25 the nucleotide sequence such that it can hybridize to the nucleotide sequence, thereby forming a stable duplex.

[0092] The nucleic acid molecule of the invention,
30 moreover, can comprise only a portion of the nucleic acid sequence of a marker nucleic acid of the invention, or a gene encoding a marker polypeptide of the invention, for example, a fragment which can be used as a probe or primer. The probe/primer typically

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comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7 or 15, preferably about 5 20 or 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 400 or more consecutive nucleotides of a marker nucleic acid, or a nucleic acid encoding a marker polypeptide of the invention.

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[0093] Probes based on the nucleotide sequence of a marker gene or of a nucleic acid molecule encoding a marker polypeptide of the invention can be used to detect transcripts or genomic sequences corresponding 15 to the marker gene(s) and/or marker polypeptide(s) of the invention. In preferred embodiments, the probe comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such 20 probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress (e.g., over- or under-express) a marker polypeptide of the invention, or which have greater or fewer copies of a marker gene of the invention. For example, a level of 25 a marker polypeptide-encoding nucleic acid in a sample of cells from a subject may be detected, the amount of mRNA transcript of a gene encoding a marker polypeptide may be determined, or the presence of mutations or deletions of a marker gene of the invention may be 30 assessed.

[0094] The invention further encompasses nucleic acid molecules that differ from the nucleic acid sequences of the genes set forth in Tables 1-5 or 7-10, due to 35 degeneracy of the genetic code and which thus encode

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the same proteins as those encoded by the genes shown in Tables 1-5 or 7-10.

[0095] In addition to the nucleotide sequences of the genes set forth in Tables 1-5 or 7-10, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the proteins encoded by the genes set forth in Tables 1-5 or 7-10 may exist within a population *e.g.*, the human population). Such genetic polymorphism in the genes set forth in Tables 1-5 or 7-10 may exist among individuals within a population due to natural allelic variation. An allele is one of a group of genes which occur alternatively at a given genetic locus. In addition it will be appreciated that DNA polymorphisms that affect RNA expression levels can also exist that may affect the overall expression level of that gene *e.g.*, by affecting regulation or degradation). As used herein, the phrase "allelic variant" includes a nucleotide sequence which occurs to a given locus or to a polypeptide encoded by the nucleotide sequence. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a marker polypeptide of the invention.

[0096] Nucleic acid molecules corresponding to natural allelic variants and homologues of the marker genes, or genes encoding the marker proteins of the invention can be isolated based on their homology to the genes set forth in Tables 7-10, using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural allelic variants and

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homologues of the marker genes of the invention can further be isolated by mapping to the same chromosome or locus as the marker genes or genes encoding the marker proteins of the invention.

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[0097] In another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 10 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule corresponding to a nucleotide sequence of a marker gene or gene encoding a marker protein of the invention. As 15 used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Preferably, 20 the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the 25 art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of one of the genes set forth in Tables 1- 30 5 or 7-10 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule includes an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

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[0098] In addition to naturally-occurring allelic variants of the marker gene and gene encoding a marker protein of the invention sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of the marker genes or genes encoding the marker proteins of the invention, thereby leading to changes in the amino acid sequence of the encoded proteins, without altering the functional activity of these proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of a protein without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among allelic variants or homologs of a gene (e.g., among homologs of a gene from different species) are predicted to be particularly unamenable to alteration.

[0099] Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding a marker protein of the invention that contain changes in amino acid residues that are not essential for activity. Such proteins differ in amino acid sequence from the marker proteins encoded by the genes set forth in Tables 1-5 or 7-10, yet retain biological activity. In one embodiment, the protein comprises an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to a marker protein of the invention.

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[0100] An isolated nucleic acid molecule encoding a protein homologous to a marker protein of the invention can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of the gene encoding the marker protein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into the genes of the invention (e.g., a gene set forth in Tables 7-10) by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of a coding sequence of a gene of the invention, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the

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encoded protein can be expressed recombinantly and the activity of the protein can be determined.

[0101] Another aspect of the invention pertains to
5 isolated nucleic acid molecules which are antisense to the marker genes and genes encoding marker proteins of the invention. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to
10 the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire coding strand of a gene of
15 the invention (*e.g.*, a gene set forth in Tables 1-5 or 7-10), or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence of the invention. The term
20 "coding region" includes the region of the nucleotide sequence comprising codons which are translated into amino acid. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence
25 of the invention.

[0102] The term "noncoding region" includes 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as
30 5' and 3' untranslated regions).

[0103] Antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can

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be complementary to the entire coding region of an mRNA corresponding to a gene of the invention, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art.

For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid

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methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically
5 using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following
10 subsection).

[0104] The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind
15 to cellular mRNA and/or genomic DNA encoding a marker protein of the invention to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a
20 stable duplex, or, for example, in the cases of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of
25 the invention include direct injection at a tissue site (*e.g.*, in brain). Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be
30 modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid
35 molecules can also be delivered to cells using the

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vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

[0105] In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

[0106] In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhof and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave mRNA transcripts of the genes of the invention (e.g., a gene set forth in Tables 1-5 or 7-10) to thereby inhibit translation of this mRNA. A ribozyme having specificity for a marker protein-encoding nucleic acid can be designed based upon the nucleotide sequence of a gene of the invention, disclosed herein. For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which

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the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a marker protein-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, mRNA transcribed from a gene of the invention can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

10 [0107] Alternatively, expression of a gene of the invention (e.g., a gene set forth in Tables 1-5 or 7-10) can be inhibited by targeting nucleotide sequences complementary to the regulatory region of these genes (e.g., the promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al. (1992). *Ann. N. Y. Acad Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

20 [0108] Expression of the marker genes, and genes encoding marker proteins of the invention, can also be inhibited using RNA interference ("RNA_i"). This is a technique for post transcriptional gene silencing ("PTGS"), in which target gene activity is specifically abolished with cognate double-stranded RNA ("dsRNA"). RNA_i resembles in many aspects PTGS in plants and has been detected in many invertebrates including

30 trypanosome, hydra, planaria, nematode and fruit fly (*Drosophila melanogaster*). It may be involved in the modulation of transposable element mobilization and antiviral state formation. RNA_i in mammalian systems is disclosed in PCT application WO 00/63364 which is

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incorporated by reference herein in its entirety. Basically, dsRNA of at least about 600 nucleotides, homologous to the target marker is introduced into the cell and a sequence specific reduction in gene activity
5 is observed. See generally, Ui-Teia, K. et al. *FEBS Letters* 479: 79-82.

[0109] In yet another embodiment, the nucleic acid molecules of the present invention can be modified at
10 the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic
15 acids (see Hyrup B. et al. (1996) *Bioorganic & Medicinal Chemistry* 4(1): 5 23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a
20 pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using
25 standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1996) *supra*; Perry-O'Keefe et al. *Proc. Natl. Acad. Sci.* 93: 14670-675.

[0110] PNAs can be used in therapeutic and diagnostic
30 applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of the nucleic acid molecules of the

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invention (e.g., a gene set forth in Tables 1-5 or 7-10) can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when
5 used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al. (1996) *supra*; Perry-O'Keefe *supra*).

10 [0111] In another embodiment, PNAs can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in
15 the art. For example, PNA-DNA chimeras of the nucleic acid molecules of the invention can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNase H and DNA polymerases), to interact with the DNA
20 portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*).

25 The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. et al. (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling
30 chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. et al. (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to

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produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. et al. (1996) *supra*).

Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. et al. (1975) *Bioorganic Med Chem. Lett.* 5: 1119-11124).

[0112] In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre et al. (1987) *Pros. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent). Finally, the oligonucleotide may be detectably labeled, either such that the label is detected by the addition of another reagent (e.g., a substrate for an enzymatic label), or is detectable immediately upon hybridization of the nucleotide (e.g., a radioactive label or a fluorescent label (e.g., a molecular beacon, as described in U.S. Patent 5,876,930).

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II. Isolated Proteins and Antibodies

[0113] One aspect of the invention pertains to isolated marker proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-marker protein antibodies. In one embodiment, native marker proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, marker proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a marker protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

15 [0114] An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the marker protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of marker protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of marker protein having less than about 30% (by dry weight) of non-marker protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-marker protein, still more preferably less than about 10% of non-marker protein, and most preferably less than about 5% non-marker protein. When the marker protein or

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produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

[0115] The language "substantially free of chemical precursors or other chemicals" includes preparations of marker protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of protein having less than about 30% (by dry weight) of chemical precursors or non-protein chemicals, more preferably less than about 20% chemical precursors or non-protein chemicals, still more preferably less than about 10% chemical precursors or non-protein chemicals, and most preferably less than about 5% chemical precursors or non-protein chemicals.

[0116] As used herein, a "biologically active portion" of a marker protein includes a fragment of a marker protein comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the marker protein, which include fewer amino acids than the full length marker proteins, and exhibit at least one activity of a marker protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the marker protein. A biologically active portion of a marker protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a marker protein can be used as targets for

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developing agents which modulate a marker protein-mediated activity.

[0117] In a preferred embodiment, marker protein is encoded by a gene set forth in Tables 1-5 or 7-10. In other embodiments, the marker protein is substantially homologous to a marker protein encoded by a gene set forth in Tables 1-5 or 7-10, and retains the functional activity of the marker protein, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the marker protein is a protein which comprises an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to the amino acid sequence encoded by a gene set forth in Tables 1-5 or 7-10.

[0118] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in

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the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two
5 sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

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[0119] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two
15 amino acid sequences is determined using the Needleman and Wunsch (*J. Mot. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossom 62 matrix
20 or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package
25 (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using
30 the algorithm of E. Meyers and W. Miller (*CABIOS*, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM 120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

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[0120] The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or
5 related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain
10 nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to marker protein molecules of the
15 invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs
20 (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

[0121] The invention also provides chimeric or fusion marker proteins. As used herein, a marker "chimeric
25 protein" or "fusion protein" comprises a marker polypeptide operatively linked to a non-marker polypeptide. An "marker polypeptide" includes a polypeptide having an amino acid sequence encoded by a gene set forth in Tables 1-5 or 7-10, whereas a "non-
30 marker polypeptide" includes a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the marker protein, e.g., a protein which is different from marker protein and which is derived from the same or a different

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organism. Within a marker fusion protein the polypeptide can correspond to all or a portion of a marker protein. In a preferred embodiment, a marker fusion protein comprises at least one biologically
5 active portion of a marker protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the marker polypeptide and the non-marker polypeptide are fused in-frame to each other. The non-marker polypeptide can be fused to the N-terminus or C-
10 terminus of the marker polypeptide.

[0122] For example, in one embodiment, the fusion protein is a GST-marker fusion protein in which the marker sequences are fused to the C-terminus of the GST
15 sequences. Such fusion proteins can facilitate the purification of recombinant marker proteins.

[0123] In another embodiment, the fusion protein is a marker protein containing a heterologous signal
20 sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of marker proteins can be increased through use of a heterologous signal sequence. Such signal sequences are well known in the art.

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[0124] The marker fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject in vivo, as described herein. The marker fusion proteins can be used to affect the
30 bioavailability of a marker protein substrate. Use of marker fusion proteins may be useful therapeutically for the treatment of disorders (e.g., multiple sclerosis) caused by, for example, (i) aberrant modification or mutation of a gene encoding a marker

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protein; (ii) mis-regulation of the marker protein-encoding gene; and (iii) aberrant post-translational modification of a marker protein.

5 [0125] Moreover, the marker-fusion proteins of the invention can be used as immunogens to produce anti-marker protein antibodies in a subject, to purify marker protein ligands and in screening assays to identify molecules which inhibit the interaction of a
10 marker protein with a marker protein substrate.

[0126] Preferably, a marker chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for
15 the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-
20 in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively,
25 PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example,
30 *Current Protocols In Molecular Biology*, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A marker protein-encoding nucleic acid
35 can be cloned into such an expression vector such that

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the fusion moiety is linked in-frame to the marker protein.

[0127] A signal sequence can be used to facilitate
5 secretion and isolation of the secreted protein or
other proteins of interest. Signal sequences are
typically characterized by a core of hydrophobic amino
acids which are generally cleaved from the mature
protein during secretion in one or more cleavage
10 events. Such signal peptides contain processing sites
that allow cleavage of the signal sequence from the
mature proteins as they pass through the secretory
pathway. Thus, the invention pertains to the described
polypeptides having a signal sequence, as well as to
15 polypeptides from which the signal sequence has been
proteolytically cleaved (*i.e.*, the cleavage products).
In one embodiment, a nucleic acid sequence encoding a
signal sequence can be operably linked in an expression
vector to a protein of interest, such as a protein
20 which is ordinarily not secreted or is otherwise
difficult to isolate. The signal sequence directs
secretion of the protein, such as from a eukaryotic
host into which the expression vector is transformed,
and the signal sequence is subsequently or concurrently
25 cleaved. The protein can then be readily purified from
the extracellular medium by art recognized methods.

[0128] Alternatively, the signal sequence can be
linked to the protein of interest using a sequence
30 which facilitates purification, such as with a GST
domain.

[0129] The present invention also pertains to variants
of the marker proteins of the invention which function

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as either agonists (mimetics) or as antagonists to the marker proteins. Variants of the marker proteins can be generated by mutagenesis, e.g., discrete point mutation or truncation of a marker protein. An agonist
5 of the marker proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a marker protein. An antagonist of a marker protein can inhibit one or more of the activities of the naturally occurring form of
10 the marker protein by, for example, competitively modulating an activity of a marker protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant
15 having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the marker protein.

20 [0130] Variants of a marker protein which function as either marker protein agonists (mimetics) or as marker protein antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a marker protein for marker protein agonist
25 or antagonist activity. In one embodiment, a variegated library of marker protein variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of marker protein variants can be
30 produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential marker protein sequences is expressible as individual polypeptides, or alternatively, as a set of larger
35 fusion proteins (e.g., for phage display) containing

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the set of marker protein sequences therein. There are a variety of methods which can be used to produce libraries of potential marker protein variants from a degenerate oligonucleotide sequence. Chemical
5 synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the
10 sequences encoding the desired set of potential marker protein sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1055; Ike et al. (1983) *Nucleic Acid Res.* 11:477).

[0131] In addition, libraries of fragments of a protein coding sequence corresponding to a marker
20 protein of the invention can be used to generate a variegated population of marker protein fragments for screening and subsequent selection of variants of a marker protein. In one embodiment, a library of coding sequence fragments can be generated by treating a
25 double stranded PCR fragment of a marker protein coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense
30 pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-

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terminal, C-terminal and internal fragments of various sizes of the marker protein.

[0132] Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are amenable to high-throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify marker variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

[0133] An isolated marker protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind marker proteins using standard techniques for polyclonal and monoclonal antibody preparation. A full-length marker protein can be used or, alternatively, the invention provides antigenic peptide fragments of these proteins for use as immunogens. The antigenic peptide of a marker protein comprises at least 8 amino acid residues of an amino acid sequence encoded by a gene set forth in Tables 1-5 or 7-10, and encompasses an epitope of a

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marker protein such that an antibody raised against the peptide forms a specific immune complex with the marker protein. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

[0134] Preferred epitopes encompassed by the antigenic peptide are regions of the marker protein that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

[0135] A marker protein immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed marker protein or a chemically synthesized marker polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic marker protein preparation induces a polyclonal anti-marker protein antibody response.

[0136] Accordingly, another aspect of the invention pertains to anti-marker protein antibodies. The term "antibody" as used herein includes immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a marker

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protein. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind to marker proteins. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, includes a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope. A monoclonal antibody composition thus typically displays a single binding affinity for a particular marker protein with which it immunoreacts.

[0137] Polyclonal anti-marker protein antibodies can be prepared as described above by immunizing a suitable subject with a marker protein of the invention. The anti-marker protein antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized marker protein. If desired, the antibody molecules directed against marker proteins can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography, to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-marker protein antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown et al. (1981) *J. Immunol.* 127:539-46; Brown et al. (1980) *J. Biol. Chem.* 255:4980-83; Yeh et al.

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(1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh et al. (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole et al. (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M.L. Gefter et al. (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a marker protein immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds to a marker protein of the invention.

[0138] Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-marker protein monoclonal antibody (see, e.g., G. Galfre et al. (1977) *Nature* 266:SSOS2; Gefter et al. *Somatic Cell Genet.*, cited *supra*; Letter, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing

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lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, axninopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp210-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind to a marker protein, e.g., using a standard ELISA assay.

[0139] Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-marker protein antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phase display library) with marker protein to thereby isolate immunoglobulin library members that bind to a marker protein. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and

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- reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. PCT International Publication No. WO 92/18619; Dower et al. PCT International Publication No. WO 91/17271; Winter et al. PCT International Publication WO 92/20791; Markland et al. PCT International Publication No. WO 92115679; Breitling et al. PCT International Publication WO 93/01288;
- 10 McCafferty et al. PCT International Publication No. WO 92/01047; Garrard et al. PCT International Publication No. WO 92/09690; Ladner et al. PCT International Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum. Antibod Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J. Mol. Biol.* 226:889-896; Clarkson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580;
- 20 Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty et al. *Nature* (1990) 348:552-554.

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- [0140] Additionally, recombinant anti-marker protein antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention.
- 30 Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al. International Application No. PCT/US86/02269;

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- Akira, et al. European Patent Application 184,187;
Taniguchi, M., European Patent Application 171,496;
Morrison et al. European Patent Application 173,494;
Neuberger et al. PCT International Publication No.
5 WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567;
Cabilly et al. European Patent Application 125,023;
Better et al. (1988) *Science* 240:1041-1043; Liu et al.
(1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu et
al. (1987) *J. Immunol.* 139:3521-3526; Sun et al. (1987)
10 *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura et al.
(1987) *Canc. Res.* 47:999-1005; Wood et al. (1985)
Nature 314:446-449; and Shaw et al. (1988) *J. Natl.
Cancer Inst.* 80:1553-1559; Morrison, S.L. (1985)
Science 229:1202-1207; Oi et al. (1986) *BioTechniques*
15 4:214; Winter U.S. Patent 5,225,539; Jones et al.
(1986) *Nature* 321:552-525; Verhoeyan et al. (1988)
Science 239:1534; and Beidler et al. (1988) *J. Immunol.*
141:4053-4060.
- 20 [0141] Humanized antibodies are particularly desirable
for therapeutic treatment of human subjects. Humanized
forms of non-human (e.g. murine) antibodies are
chimeric molecules of immunoglobulins, immunoglobulin
chains or fragments thereof (such as Fv, Fab, Fab',
25 F(ab')₂, or other antigen-binding subsequences of
antibodies) which contain minimal sequence derived from
non-human immunoglobulin. Humanized antibodies include
human immunoglobulins (recipient antibody) in which
residues forming a complementary determining region
30 (CDR) of the recipient are replaced by residues from a
CDR of a non-human species (donor antibody) such as
mouse, rat or rabbit having the desired specificity,
affinity and capacity. In some instances, Fv framework
residues of the human immunoglobulin are replaced by

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corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the constant regions being those of a human immunoglobulin consensus sequence. The humanized antibody will preferably also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al. *Nature* 321: 522-525 (1986); Riechmann et al, *Nature* 323: 323-329 (1988); and Presta *Curr.Op.Struct.Biol.* 2: 594-596 (1992).

[0142] Such humanized antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide corresponding to a marker of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE antibodies. For an overview of this technology for producing humanized antibodies, see Lonberg and Huszar (1995) *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for

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producing humanized antibodies and humanized monoclonal antibodies and protocols for producing such antibodies, see, e.g., U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA), can be engaged to provide humanized antibodies directed against a selected antigen using technology similar to that described above.

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[0143] Humanized antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a murine antibody, is used to guide the selection of a humanized antibody recognizing the same epitope (Jespers et al., 1994, *Bio/technology* 12:899-903).

[0144] An anti-marker protein antibody (e.g., monoclonal antibody) can be used to isolate a marker protein of the invention by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-marker protein antibody can facilitate the purification of natural marker proteins from cells and of recombinantly produced marker proteins expressed in host cells. Moreover, an anti-marker protein antibody can be used to detect marker protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the marker protein. Anti-marker protein antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling

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(i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

20 III. Recombinant Expression Vectors and Host Cells

[0145] Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a marker protein of the invention (or a portion thereof). As used herein, the term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which includes a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and

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episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host
5 genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are
10 often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression
15 vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

[0146] The recombinant expression vectors of the
20 invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for
25 expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequences) in a manner which allows
30 for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control
35 elements (e.g., polyadenylation signals). Such

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regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct

5 constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in

10 the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby

15 produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., marker proteins, mutant forms of marker proteins, fusion proteins, and the like).

20 [0147] The recombinant expression vectors of the invention can be designed for expression of marker proteins in prokaryotic or eukaryotic cells. For example, marker proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus

25 expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed

30 and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[0148] Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing

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constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRITS (Pharmacia, Piscataway, NJ) which fuse glutathione S transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

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[0149] Purified fusion proteins can be utilized in marker activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for marker proteins, for example.

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[0150] Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Hmann et al., (1988) *Gene* 69:301-315) and pET 11d (Studier et al.,

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Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

[0151] One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wade et al., (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

[0152] In another embodiment, the marker protein expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, et al., (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., 1987) *Gene*

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54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

[0153] Alternatively, marker proteins of the invention
5 can be expressed in insect cells using baculovirus
expression vectors. Baculovirus vectors available for
expression of proteins in cultured insect cells (e.g.,
Sf 9 cells) include the pAc series (Smith et al. (1983)
Mol. Cell Biol. 3:2156-2165) and the pVL series
10 (Lucklow and Summers (1989) *Virology* 170:31-39).

[0154] In yet another embodiment, a nucleic acid of
the invention is expressed in mammalian cells using a
mammalian expression vector. Examples of mammalian
15 expression vectors include pCDM8 (Seed, B. (1987)
Nature 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO*
J. 6:187-195). When used in mammalian cells, the
expression vector's control functions are often
provided by viral regulatory elements. For example,
20 commonly used promoters are derived from polyoma,
Adenovirus 2, cytomegalovirus and Simian Virus 40. For
other suitable expression systems for both prokaryotic
and eukaryotic cells see chapters 16 and 17 of
Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular*
25 *Cloning: A Laboratory Manual*. 2nd, ed.. Cold Spring
Expression Technology: Methods in Enzymology 185,
Academic Press, San Diego, California (1990) 60-89).
Target gene expression from the pTrc vector relies on
host RNA polymerase transcription from a hybrid trp-lac
30 fusion promoter. Target gene expression from the pET
11d vector relies on transcription from a T7 gn10-lac
fusion promoter mediated by a coexpressed viral RNA
polymerase (T7 gn1). This viral polymerase is supplied
by host strains BL21(DE3) or HMS174(DE3) from a

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resident prophage harboring a T7 *gn1* gene under the transcriptional control of the *lacUV 5* promoter.

[0155] One strategy to maximize recombinant protein
5 expression in *E. coli* is to express the protein in a
host bacteria with an impaired capacity to
proteolytically cleave the recombinant protein
(Gottesman, S., *Gene Expression Technology: Methods in
Enzymology* 185, Academic Press, San Diego, California
10 (1990) 119-128). Another strategy is to alter the
nucleic acid sequence of the nucleic acid to be
inserted into an expression vector so that the
individual codons for each amino acid are those
preferentially utilized in *E. coli* (Wada et al., (1992)
15 *Nucleic Acids Res.* 20:2111-2118). Such alteration of
nucleic acid sequences of the invention can be carried
out by standard DNA synthesis techniques.

[0156] In another embodiment, the marker protein
20 expression vector is a yeast expression vector.
Examples of vectors for expression in yeast *S.
cerevisiae* include pYepSec1 (Baldari, et al., (1987)
Embo J. 6:229-234), pMFa (Kurjan and Herskowitz, (1982)
Cell 30:933-943), pJRY88 (Schultz et al., (1987) *Gene*
25 54:113-123), pYES2 (Invitrogen Corporation, San Diego,
CA), and *picZ* (Invitrogen Corp, San Diego, CA).

[0157] Alternatively, marker proteins of the invention
can be expressed in insect cells using baculovirus
30 expression vectors. Baculovirus vectors available for
expression of proteins in cultured insect cells (e.g.,
Sf9 cells) include the pAc series (Smith et al. (1983)
Mol. Cell Biol. 3:2156-2165) and the pVL series
(Lucklow and Summers (1989) *Virology* 170:31-39).

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[0158] In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

[0159] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter, Byrne and R.aaddle (1989) *Proc.*

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Natl. Acad Sci. USA 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter, U.S. Patent No. 4,873,316 and European
5 Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the marine hox promoters (Kessel and Grass (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

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[0160] The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is
15 operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to mRNA corresponding to a gene of the invention (e.g., a gene set forth in Tables 1-5 or 7-10). Regulatory
20 sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can
25 be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the
30 control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a

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molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1)1986.

[0161] Another aspect of the invention pertains to
5 host cells into which a nucleic acid molecule of the
invention is introduced, e.g., a gene set forth in
Tables 1-5 or 7-10 within a recombinant expression
vector or a nucleic acid molecule of the invention
containing sequences which allow it to homologously
10 recombine into a specific site of the host cell's
genome. The terms "host cell" and "recombinant host
cell" are used interchangeably herein. It is
understood that such terms refer not only to the
particular subject cell but to the progeny or potential
15 progeny of such a cell. Because certain modifications
may occur in succeeding generations due to either
mutation or environmental influences, such progeny may
not, in fact, be identical to the parent cell, but are
still included within the scope of the term as used
20 herein.

[0162] A host cell can be any prokaryotic or
eukaryotic cell. For example, a marker protein of the
invention can be expressed in bacterial cells such as
25 *E. coli*, insect cells, yeast or mammalian cells (such
as Chinese hamster ovary cells (CHO) or COS cells).
Other suitable host cells are known to those skilled in
the art.

30 [0163] Vector DNA can be introduced into prokaryotic
or eukaryotic cells via conventional transformation or
transfection techniques. As used herein, the terms
"transformation" and "transfection" are intended to
refer to a variety of art-recognized techniques for

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introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transferring host cells can be found in Sambrook, et al. (*Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

10

[0164] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable flag (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable flags include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable flag can be introduced into a host cell on the same vector as that encoding a marker protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable flag gene will survive, while the other cells die).

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[0165] A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) a marker protein. Accordingly, the invention further provides methods for producing a marker protein using the host cells of the

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invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a marker protein has been introduced) in a suitable medium such that a
5 marker protein of the invention is produced. In another embodiment, the method further comprises isolating a marker protein from the medium or the host cell.

10 [0166] The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which marker-protein-coding sequences have
15 been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous sequences encoding a marker protein of the invention have been introduced into their genome or homologous recombinant animals in which endogenous sequences
20 encoding the marker proteins of the invention have been altered. Such animals are useful for studying the function and/or activity of a marker protein and for identifying and/or evaluating modulators of marker protein activity. As used herein, a "transgenic
25 animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats,
30 chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in
35 one or more cell types or tissues of the transgenic

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animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous gene of the invention (e.g., a gene set forth in Tables 1-5 or
5 7-10) has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

10

[0167] A transgenic animal of the invention can be created by introducing a marker-encoding nucleic acid into the mate pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing
15 the oocyte to develop in a pseudopregnant female foster animal. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked
20 to a transgene to direct expression of a marker protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example,
25 in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Patent No. 4,873,191 by Wagner et al. and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for
30 production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a transgene of the invention in its genome and/or expression of mRNA corresponding to a gene of the invention in tissues or cells of the animals. A
35 transgenic founder animal can then be used to breed

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additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a marker protein can further be bred to other transgenic animals carrying other transgenes.

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[0168] To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a gene of the invention into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the gene. The gene can be a human gene, but more preferably, is a non-human homologue of a human gene of the invention (e.g., a gene set forth in Tables 1-5 or 7-10). For example, a mouse gene can be used to construct a homologous recombination nucleic acid molecule, e.g., a vector, suitable for altering an endogenous gene of the invention in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous gene of the invention is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous marker protein). In the homologous recombination nucleic acid molecule, the altered portion of the gene of the invention is flanked at its 5' and 3' ends by additional nucleic acid sequence of the gene of the invention to allow for homologous recombination to occur between the exogenous gene carried by the homologous recombination nucleic acid

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molecule and an endogenous gene in a cell, e.g., an embryonic stem cell. The additional flanking nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene.

5 Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, e.g., Thomas, K.R. and Capecchi, M.R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The

10 homologous recombination nucleic acid molecule is introduced into a cell, e.g., an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced gene has homologously recombined with the endogenous gene are selected (see e.g., Li, E. et al.

15 (1992) *Cell* 69:915). The selected cells can then be injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see e.g. Bradley, S A. in *Teratocareirtomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford,

20 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells

25 of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, e.g., vectors, or homologous recombinant animals are described further in Bradley, A. (1991)

30 *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec et al.; WO 91/01140 by Smithies et al.; WO 92/0968 by Zijlstra et al.; and WO 93/04169 by Berns et al.

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[0169] In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene.

5 One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, e.g., Laksa et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system

10 is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et al. (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase

15 and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a

20 recombinase.

[0170] Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. et al. (1997) *Nature*

25 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, e.g.,

30 through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant

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female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

5 IV. Pharmaceutical Compositions

[0171] The nucleic acid molecules of the invention (e.g., the genes set forth in Tables 1-5 or 7-10), fragments of marker proteins, and anti-marker protein
10 antibodies of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions (also referred to herein as "bioactive agents or compounds") typically comprise the nucleic acid molecule, protein, or
15 antibody and a pharmaceutically acceptable carrier.

[0172] As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and
20 antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well-known in the art. Except insofar as any conventional media or agent
25 is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary bioactive agents can also be incorporated into the compositions.

30 [0173] The invention includes methods for preparing pharmaceutical compositions for modulating the expression or activity of a polypeptide or nucleic acid corresponding to a marker of the invention. Such methods comprise formulating a pharmaceutically

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acceptable carrier with an agent which modulates expression or activity of a polypeptide or nucleic acid corresponding to a marker of the invention. Such compositions can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a pharmaceutically acceptable carrier with an agent which modulates expression or activity of a polypeptide or nucleic acid corresponding to a marker of the invention and one or more additional bioactive agents.

[0174] The invention also provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents comprising therapeutic moieties (e.g., peptides, peptidomimetics, peptoids, small molecules or other drugs) which (a) bind to the marker, or (b) have a modulatory (e.g., stimulatory or inhibitory) effect on the activity of the marker or, more specifically, (c) have a modulatory effect on the interactions of the marker with one or more of its natural substrates (e.g., peptide, protein, hormone, co-factor, or nucleic acid), or (d) have a modulatory effect on the expression of the marker. Such assays typically comprise a reaction between the marker and one or more assay components. The other components may be either the test compound itself, or a combination of test compound and a natural binding partner of the marker.

[0175] The test compounds of the present invention may be bioactive agents, i.e. protein, oligopeptide, molecule, polysaccharide, polynucleotides. In a preferred embodiment the bioactive agents are proteins,

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in particular naturally occurring proteins or fragments thereof.

[0176] The test compounds of the present invention may
5 be obtained from any available source, including
systematic libraries of natural and/or synthetic
compounds. Test compounds may also be obtained by any
of the numerous approaches in combinatorial library
methods known in the art, including: biological
10 libraries; peptoid libraries (libraries of molecules
having the functionalities of peptides, but with a
novel, non-peptide backbone which are resistant to
enzymatic degradation but which nevertheless remain
bioactive; see, e.g., Zuckermann et al., 1994, *J. Med.*
15 *Chem.* 37:2678-85); spatially addressable parallel solid
phase or solution phase libraries; synthetic library
methods requiring deconvolution; the 'one-bead one-
compound' library method; and synthetic library methods
using affinity chromatography selection. The
20 biological library and peptoid library approaches are
limited to peptide libraries, while the other four
approaches are applicable to peptide, non-peptide
oligomer or small molecule libraries of compounds (Lam,
1997, *Anticancer Drug Des.* 12:145).

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[0177] A pharmaceutical composition of the invention
is formulated to be compatible with its intended route
of administration. Examples of routes of
administration include parenteral, e.g., intravenous,
30 intradermal, subcutaneous, oral (e.g., inhalation),
transdermal (topical), transmucosal, and rectal
administration. Solutions or suspensions used for
parenteral, intradermal, or subcutaneous application
can include the following components: a sterile

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diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine; propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens;

5 antioxidants such as ascorbic acid or sodium bisulfate; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with

10 acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

15 [0178] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous

20 administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists.

25 It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol

30 (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required

35 particle size in the case of dispersion and by the use

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of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0179] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a fragment of a marker protein or an anti-marker protein antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

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[0180] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used

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in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and
5 expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar
10 nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Stertes; a glidant such as
15 colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0181] For administration by inhalation, the compounds
20 are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

25 [0182] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in
30 the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the

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bioactive compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0183] The compounds can also be prepared in the form
5 of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0184] In one embodiment, the therapeutic moieties,
10 which may contain a bioactive compound, are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable,
15 biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials
20 can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These
25 can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[0185] It is especially advantageous to formulate oral
30 or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein includes physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity

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of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and
5 directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

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[0186] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50%
15 of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large
20 therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected
25 cells and, thereby, reduce side effects.

[0187] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such
30 compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in
35 the method of the invention, the therapeutically

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effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0188] The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

[0189] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

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V. Computer Readable Means and Arrays

[0190] Computer readable media comprising a marker(s) of the present invention is also provided. As used
5 herein, "computer readable media" includes a medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such
10 as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. The skilled artisan will readily appreciate how any of the presently known computer readable mediums can be used to create a
15 manufacture comprising computer readable medium having recorded thereon a marker of the present invention.

[0191] As used herein, "recorded" includes a process for storing information on computer readable medium.
20 Those skilled in the art can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising the markers of the present invention.

25 [0192] A variety of data processor programs and formats can be used to store the marker information of the present invention on computer readable medium. For example, the nucleic acid sequence corresponding to the markers can be represented in a word processing text
30 file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. Any number of dataprocessor structuring formats (e.g.,

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text file or database) may be adapted in order to obtain computer readable medium having recorded thereon the markers of the present invention.

5 [0193] By providing the markers of the invention in computer readable form, one can routinely access the marker sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid sequences of the invention in
10 computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target
15 sequence or target motif.

[0194] The invention also includes an array comprising a marker(s) of the present invention, i.e. a biochip. The array can be used to assay expression of one or
20 more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 8600 genes can be simultaneously assayed for expression. This allows an
25 expression profile to be developed showing a battery of genes specifically expressed in one or more tissues at a given point in time.

[0195] In addition to such qualitative determination,
30 the invention allows the quantitation of gene expression in the biochip. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue
35 expression per se and level of expression in that

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tissue. As used herein, a "normal level of expression" refers to the level of expression of a gene provided in a control sample, typically the control is from non-involved cells or tissues, or from a non-diseased
5 subject. The determination of normal levels of expression is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be
10 determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an
15 agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a
20 counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be
25 ascertained and counteracted.

[0196] In another embodiment, the arrays can be used to monitor the time course of expression of one or more genes in the array. This can occur in various
30 biological contexts, as disclosed herein, for example development and differentiation, disease progression, in vitro processes, such a cellular transformation and senescence, autonomic neural and neurological processes, such as, for example, pain and appetite, and
35 cognitive functions, such as learning or memory.

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[0197] The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells.

5 This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

10 [0198] The array is also useful for ascertaining differential expression patterns of one or more genes in non-involved or diseased cells. This provides a battery of genes that could serve as a molecular target for diagnosis or therapeutic intervention. In
15 particular, biochips can be made comprising arrays not only of the differentially expressed markers listed in Tables 1-5 or 7-10, but of markers specific to subjects at a certain stage of the disease (i.e. secondary progressive, primary progressive, relapsing-remitting),
20 or from higher-risk geographical regions (i.e. northern Europe or Canada).

VI. Predictive Medicine

25 [0199] The present invention pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenetics and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual
30 prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining marker protein and/or nucleic acid expression as well as marker protein activity, in the context of a biological sample (e.g., blood, serum,

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cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with increased or decreased marker protein expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with marker protein, nucleic acid expression or activity. For example, the number of copies of a marker gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purposes to thereby prophylactically treat an individual prior to the onset of a disorder (e.g., multiple sclerosis) characterized by or associated with marker protein, nucleic acid expression or activity.

[0200] Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of marker in clinical trials.

[0201] These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

[0202] An exemplary method for detecting the presence or absence of marker protein or nucleic acid of the invention in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting the protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes the marker protein such that the presence of the marker protein or nucleic acid

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is detected in the biological sample. A preferred agent for detecting mRNA or genomic DNA corresponding to a marker gene or protein of the invention is a labeled nucleic acid probe capable of hybridizing to a mRNA or genomic DNA of the invention. Suitable probes for use in the diagnostic assays of the invention are described herein.

[0203] A preferred agent for detecting marker protein is an antibody capable of binding to marker protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect marker mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of marker mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of marker protein include enzyme linked immunosorbent assays (ELISAs),

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Western blots, immunoprecipitations and immunofluorescence. In vitro techniques for detection of marker genomic DNA include Southern hybridizations. Furthermore, in vivo techniques for detection of marker protein include introducing into a subject a labeled anti-marker antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

10

[0204] In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

[0205] In another embodiment, the methods further involve obtaining a control biological sample (e.g., noninvolved tissue or from a non-diseased subject) from a control subject, contacting the control sample with a compound or agent capable of detecting marker protein, mRNA, or genomic DNA, such that the presence of marker protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of marker protein, mRNA or genomic DNA in the control sample with the presence of marker protein, mRNA or genomic DNA in the test sample.

30

[0206] The invention also encompasses kits for detecting the presence of marker in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting marker protein

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or mRNA in a biological sample; means for determining the amount of marker in the sample; and means for comparing the amount of marker in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect marker protein or nucleic acid.

2. Prognostic Assays

10

[0207] The diagnostic methods, described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant marker expression or activity. As used herein, the term "aberrant" includes a marker expression or activity which deviates from the wild type marker expression or activity. Aberrant expression or activity includes increases or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant marker expression or activity is intended to include the cases in which a mutation in the marker gene causes the marker gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional marker protein or a protein which does not function in a wild-type fashion, e.g., a protein which does not interact with a marker ligand or one which interacts with a non marker protein ligand.

[0208] The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a

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misregulation in marker protein activity or nucleic acid expression, such as multiple sclerosis. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a, disorder associated with a misregulation in marker protein activity or nucleic acid expression, such as multiple sclerosis. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant marker expression or activity in which a test sample is obtained from a subject and marker protein or nucleic acid (e.g., mRNA or genomic DNA) is detected, wherein the presence of marker protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant marker expression or activity. As used herein, a "test sample" includes a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., blood PBMCs), cell sample, or tissue (e.g., brain).

20 [0209] Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with increased or decreased marker expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder such as multiple sclerosis. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with increased or decreased marker expression or activity in which a test sample is obtained and marker protein or nucleic acid expression

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or activity is detected (e.g., wherein the abundance of marker protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with increased or decreased marker expression or activity).

[0210] The methods of the invention can also be used to detect genetic alterations in a marker gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in marker protein activity or nucleic acid expression, such as multiple sclerosis. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a marker-protein, or the mis-expression of the marker gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a marker gene; 2) an addition of one or more nucleotides to a marker gene; 3) a substitution of one or more nucleotides of a marker gene, 4) a chromosomal rearrangement of a marker gene; 5) an alteration in the level of a messenger RNA transcript of a marker gene, 6) aberrant modification of a marker gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a marker gene, 8) a non-wild type level of a marker-protein, 9) allelic loss of a marker gene, and 10) inappropriate post-translational modification of a marker-protein. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a marker gene. A preferred biological sample is a tissue (e.g.,

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brain) or blood sample isolated by conventional means from a subject.

[0211] In certain embodiments, detection of the
5 alteration involves the use of a probe/primer in a
polymerase chain reaction (PCR) (see, e.g., U.S. Patent
Nos. 4,683, (95 and 4,683,202), such as anchor PCR or
RACE PCR, or, alternatively, in a ligation chain
reaction (LCR) (see, e.g., Landegran et al. (1988)
10 *Science* 241:1077-1080; and Nakazawa et al. (1994) *Proc.*
Mail. Acad. Sci. USA 91:360-364), the latter of which
can be particularly useful for detecting point
mutations in the marker-gene (see Abravaya et al.
(1995) *Nucleic Acids Res.* 23:675-682). This method can
15 include the steps of collecting a sample of cells from
a subject, isolating nucleic acid (e.g., genomic, mRNA
or both) from the cells of the sample, contacting the
nucleic acid sample with one or more primers which
specifically hybridize to a marker gene under
20 conditions such that hybridization and amplification of
the marker-gene (if present) occurs, and detecting the
presence or absence of an amplification product, or
detecting the size of the amplification product and
comparing the length to a control sample. It is
25 anticipated that PCR and/or LCR may be desirable to use
as a preliminary amplification step in conjunction with
any of the techniques used for detecting mutations
described herein.

30 [0212] Alternative amplification methods include:
self sustained sequence replication (Guatelli, JC. et
al., (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878),
transcriptional amplification system (Kwoh, D.Y. et
al., (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177),

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Q-Beta Replicase (Lizardi, P.M. et al. (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

10 [0213] In an alternative embodiment, mutations in a marker gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more
15 restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see,
20 for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

[0214] In other embodiments, genetic mutations in a
25 marker gene or a gene encoding a marker protein of the invention can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin, M.T. et al. (1996)
30 *Human Mutation* 7: 244-255; Kozal, M.J. et al. (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in marker can be identified in two dimensional arrays containing light generated DNA probes as described in Cronin, M.T. et al. *supra*.

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Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential
5 overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all
10 variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

15 [0215] In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the marker gene and detect mutations by comparing the sequence of the sample marker with the corresponding wild-type (control) sequence. Examples
20 of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing
25 procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94116101; Cohen et al. (1996) *Adv. Chromatogr.* 36:127-162; and Griffin et
30 al. (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

[0216] Other methods for detecting mutations in the marker gene or gene encoding a marker protein of the invention include methods in which protection from

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cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes
5 by hybridizing (labeled) RNA or DNA containing the wild-type marker sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as
10 which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digest the mismatched regions. In other embodiments, either
15 DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide
20 gels to determine the site of mutation. See, for example, Cotton et al. (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba et al. (1992) *Methods Enzymol.* 517:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

25

[0217] In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined
30 systems for detecting and mapping point mutations in marker cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al. (1994)

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Carcinogenesis 15:1657-1652). According to an exemplary embodiment, a probe based on a marker sequence, e.g., a wild-type marker sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

- 10 [0218] In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in marker genes or genes encoding a marker protein of the invention. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al. (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech Appl.* 9:73-79). Single-stranded DNA fragments of sample and control marker nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) *Trends Genet* 7:5).

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[0219] In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers
5 et al. (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 by of high-melting GC-rich DNA by PCR. In a further embodiment, a
10 temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

15 [0220] Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in
20 which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) *Nature* 324:163); Saiki et al. (1989) *Proc. Natl. Acad. Sci USA* 86:6230). Such allele
25 specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

30

[0221] Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific

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amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

[0222] The methods described herein may be performed, for example, by utilizing prepackaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose subjects exhibiting symptoms or family history of a disease or illness involving a marker gene.

[0223] Furthermore, any cell type or tissue in which marker is expressed may be utilized in the prognostic assays described herein.

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3. Monitoring of Effects During Clinical Trials

[0224] Monitoring the influence of agents (e.g., drugs) on the expression or activity of a marker protein (e.g., the modulation of multiple sclerosis) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase marker gene expression, protein levels, or upregulate marker activity, can be monitored in clinical trials of subjects exhibiting decreased marker gene expression, protein levels, or downregulated marker activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease marker gene expression, protein levels, or downregulate marker activity, can be monitored in clinical trials of subjects exhibiting increased marker gene expression, protein levels, or upregulated marker activity. In such clinical trials, the expression or activity of a marker gene, and preferably, other genes that have been implicated in, for example, a marker-associated disorder (e.g., multiple sclerosis) can be used as a "read out" or markers of the phenotype of a particular cell.

[0225] For example, and not by way of limitation, genes, including marker genes and genes encoding a marker protein of the invention, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates marker activity (e.g., identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on marker-associated disorders (e.g.,

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multiple sclerosis), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of marker and other genes implicated in the marker-associated disorder, respectively. The levels of gene expression (e.g., a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of marker or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

[0226] In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a marker protein, mRNA, or genomic DNA in the pre-administration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the marker protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the marker protein, mRNA, or genomic DNA in the pre-administration sample with the marker protein, mRNA, or genomic DNA in the post

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administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of
5 marker to higher levels than detected, *i.e.*, to increase the effectiveness of the agent.

Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of marker to lower levels than detected, *i.e.* to decrease
10 the effectiveness of the agent. According to such an embodiment, marker expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

15 C. Methods of Treatment

[0227] The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk for (or susceptible to) a disorder or
20 having a disorder associated with aberrant marker expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of
25 pharmacogenomics. "Pharmacogenomics", as used herein, includes the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers
30 the study of how a subject's genes determine his or her response to a drug (*e.g.*, a subject's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic
35 treatment with either the marker molecules of the

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present invention or marker modulators according to that individual's drug response genotype.

Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to
5 subjects who will most benefit from the treatment and to avoid treatment of subjects who will experience toxic drug-related side effects.

1. Prophylactic Methods

10

[0228] In one aspect, the invention provides a method for preventing in a subject, a disease or condition (e.g., multiple sclerosis) associated with increased or decreased marker expression or activity, by

15 administering to the subject a marker protein or an agent which modulates marker protein expression or at least one marker protein activity. Subjects at risk for a disease which is caused or contributed to by increased or decreased marker expression or activity
20 can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the differential marker protein expression, such that a
25 disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of marker aberrancy (e.g., increase or decrease in expression level), for example, a marker protein, marker protein agonist or marker protein antagonist
30 agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

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2. Therapeutic Methods

[0229] Another aspect of the invention pertains to methods of modulating marker protein expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a marker protein or agent that modulates one or more of the activities of a marker protein activity associated with the cell. An agent that modulates marker protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a marker protein (e.g., a marker protein substrate), a marker protein antibody, a marker protein agonist or antagonist, a peptidomimetic of a marker protein agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more marker protein activities. Examples of such stimulatory agents include active marker protein and a nucleic acid molecule encoding marker protein that has been introduced into the cell. In another embodiment, the agent inhibits one or more marker protein activities. Examples of such inhibitory agents include antisense marker protein nucleic acid molecules, anti-marker protein antibodies, and marker protein inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a marker protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of

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agents that modulates (e.g., upregulates or downregulates) marker protein expression or activity. In another embodiment, the method involves administering a marker protein or nucleic acid molecule
5 as therapy to compensate for reduced or aberrant marker protein expression or activity.

[0230] Stimulation of marker protein activity is desirable in situations in which marker protein is
10 abnormally downregulated and/or in which increased marker protein activity is likely to have a beneficial effect. For example, stimulation of marker protein activity is desirable in situations in which a marker is downregulated and/or in which increased marker
15 protein activity is likely to have a beneficial effect. Likewise, inhibition of marker protein activity is desirable in situations in which marker protein is abnormally upregulated and/or in which decreased marker protein activity is likely to have a beneficial effect.

20

3. Pharmacogenomics

[0231] The marker protein and nucleic acid molecules of the present invention, as well as agents, inhibitors
25 or modulators which have a stimulatory or inhibitory effect on marker protein activity (e.g., marker gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) marker-
30 associated disorders (e.g., multiple sclerosis) associated with aberrant marker protein activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to

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a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a marker molecule or marker modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a marker molecule or marker modulator.

[0232] Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. et al. (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11) :983-985 and Linden, M.W. et al. (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

[0233] One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-

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wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-
5 100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically substantial number of subjects taking part in a Phase II/III drug trial to
10 identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used
15 herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease
20 associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of
25 genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

[0234] Alternatively, a method termed the "candidate
30 gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (e.g., a marker protein of the present invention), all common variants of that gene can be fairly easily identified
35 in the population and it can be determined if having

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one version of the gene versus another is associated with a particular drug response.

[0235] As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some subjects do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

[0236] Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the

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gene expression of an animal dosed with a drug (e.g., a marker molecule or marker modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

5

[0237] Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual.

10 This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a marker molecule or marker modulator, such as a modulator
15 identified by one of the exemplary screening assays described herein.

[0238] This invention is further illustrated by the following examples which should not be construed as
20 limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and Tables are incorporated herein by reference.

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EXAMPLES

EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF
MARKER cDNA IN MURINE MODEL OF EXPERIMENTAL AUTOIMMUNE
5 ENCEPHALITIS (EAE)

A. Induction of EAE in Mice

[0239] 12 SJL mice were immunized subcutaneously with
10 150 μ g injection of proteolipid protein (PLP), peptide
residues 139-151 in complete Freud's adjuvant, a
protein capable of inducing EAE in mice. After 10
days, the spleens were harvested, and splenocyte cells
were isolated and reactivated *in vitro* for 4 days with
15 5 μ g of PLP. The amplified splenocyte cells were then
injected into SJL mice at 15×10^6 cells per mouse.

B. *In Vivo* Development of EAE and Isolation of Immune
Cells and Microglial Cells

20

[0240] Development of EAE in mice was characterized by
three stages: onset, peak and recovery. Onset of EAE
in mice was characterized by a limp tail 7-8 days after
injection. Peak development of EAE in mice appeared as
25 full hind limb paralysis approximately 10-12 days after
injection, while recovery appeared as hind limb
weakness approximately 16-18 days after injection. At
each of the above 3 stages, the following numbers of
mice were sacrificed and harvested for cervical spinal
30 cord and mid-brain: 2 mice at onset, 6 at peak and 4
at recovery. To isolate infiltrating immune cells and
microglial cells, the samples were run over a percoll
gradient to remove neurons, endothelial cells and
astrocytes.

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C. Isolation of RNA

[0241] Total RNA was isolated using the RNeasy mini kit (Quiagen, Hilden, Germany). To prepare cRNA for hybridization, 5 μ g of total RNA was denatured at 70°C with T7-tagged oligo-dT primer, cooled on ice, then reverse transcribed with 200 units Superscript RT II at 50°C for 1 hour in 1x first strand buffer, 10 mM DTT and 0.5 mM of each dNTP (Gibco BRL, Gaithersburg, MD).

10 Second strand cDNA was synthesized by adding 40 units DNA pol I, 10 units E. coli DNA ligase, 2 units Rnase H, 30 μ L second strand buffer, 3 μ L 10 mM each dNTP, and water to 150 μ L final volume and incubating at 15.8°C for 2 hours. The resulting cDNA was extracted

15 once with phenol/chloroform/isoamylalcohol. CDNA was separated on a Phase Lock Gel tube at maximum speed for 2 min and precipitated with sodium acetate and 100% ethanol. The resulting pellet was washed with 80% ethanol, was dried and was resuspended in

20 diethylpyrocarbonate-treated (DEPC-treated) water.

[0242] Labeled RNA was prepared from clones containing a T7 RNA polymerase promoter site by incorporating labeled ribonucleotides in an *in vitro* transcription (IVT) reaction. Half of the purified cDNA was used for *in vitro* transcription with a T7 RNA polymerase kit, following manufacturer instructions and using an overnight 37 °C incubation, thereby incorporating biotinylated CTP and UTP. Labeled RNA was purified

30 using RNeasy columns (Quiagen). RNA was concentrated and then quantitated by spectrophotometry. Labeled RNA (13-15 μ g) was fragmented in 40 mM Tris-acetate 8.0, 100 mM potassium acetate, 30 mM magnesium acetate for 35 min at 94 C in a total volume of 40 μ L.

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D. Array Hybridization and Detection of Fluorescence

[0243] The labeled and fragmented RNA probes were diluted in 1 x MES buffer, BIO948, Bio C, B cre, 100 $\mu\text{g/ml}$ herring sperm DNA, and 50 $\mu\text{g/ml}$ acetylated BSA. New probes were pre-hybridized in a microfuge tube with glass beads at 45°C overnight to remove debris. Oligonucleotide arrays composed of approximately 11,000 murine genes (Microarray, Affymetrix, Cat Nos. SubA #510243, SubB #510244) were pre-hybridized with 1 x MES hybridization buffer at 45°C for 5 min and then insoluble material was removed by centrifugation. Pre-hybridization buffer was removed from oligo array cartridges, 200 μL probe added and cartridges were hybridized for 16 hours at 45°C at 60 rpm. After hybridization, probes were removed and the cartridges washed extensively with 6 x SSPET using a fluidics station (Affymetrix). Following hybridization, the solutions were removed, the arrays were washed with 6x SSPE-T at 22°C for 7 min, and then washed with 0.5 x SSPE-T at 40°C for 15 minutes. When biotin-labeled RNA was used, the hybridized RNA was stained with a streptavidin-phycoerythrin conjugate (Molecule Probes, Eugene, OR) prior to reading. Hybridized arrays were stained with 2 $\mu\text{g/ml}$ streptavidin-phycoerythrin in 6x SSPE-T at 40°C for 5 minutes. The arrays were read using a scanning confocal microscope made for Affymetrix by Molecular Dynamics (commercially available through Affymetrix, Santa Clara, CA). The scanner uses an argon ion laser as the excitation source, with the emission detected by a photomultiplier tube through either a 530 nm bandpass filter (fluorescein), or a 560 nm longpass filter (phycoerythrin). Nucleic acids of either sense or antisense orientations were used in hybridization

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experiments. Arrays with probes for either orientation (reverse complements of each other) are made using the same set of photolithographic masks by reversing the order of the photochemical steps and incorporating the complementary nucleotide.

E. Quantitative Analysis of Hybridization Patterns and Insensitivities

10 [0244] Following a quantitative scan of an array, or biochip, a grid is aligned to the image using the known dimensions of the array and the corner control regions as markers. The image is reduced to a simple text file containing position and intensity information using
15 software developed at Affymetrix (GENECHIP 3.0 software). This information is merged with another text file that contains information relating physical position on the array to probe sequence and the identity of the RNA and the specific part of the RNA
20 for which the oligonucleotide probe is designed. The quantitative analysis of the hybridization results involves a simple form of pattern recognition based on the assumption that, in the presence of a specific RNA, the PM probes will hybridize more strongly on average
25 than their MM partners. The number of instances in which the PM hybridization signal is larger than the MM signal is computed along with the average of the logarithm of the PM/MM ratios for each probe set. These values are used to make a decision (using a
30 predefined decision matrix) concerning the presence or absence of an RNA. To determine the quantitative RNA abundance, the average of the differences (PM minus MM) for each probe family is calculated. The advantage of the difference method is that signals from random
35 cross-hybridization contribute equally, on average, to

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the PM and MM probes, while specific hybridization contributes more to the PM probes. By averaging the pairwise differences, the real signals add constructively while the contributions from cross-hybridization tend to cancel. When assessing the differences between two different RNA samples, the hybridization signals from side-by-side experiments on identically synthesized arrays are compared directly. The magnitude of the changes in the average of the difference (PM-MM) values is interpreted by comparison with the results of spiking experiments as well as the signals observed for the internal standard bacterial and phage RNAs spiked into each sample at a known amount. Data analysis programs developed at Affymetrix, such as the GENECHIP 3.0 software, perform these operation automatically.

[0245] Over 5000 genes were expressed in the central nervous system of the murine samples. Nonetheless, distinct gene expression patterns emerged between peak disease and onset or recovery of the disease. In order to identify the most active genes in EAE development, genes were sought which revealed a pattern of increased or decreased regulation at peak, as compared to onset or recovery. The onset stage served as the baseline comparison, with the assumption that the onset stage was similar in gene expression to undifferentiated naive T cells (i.e., similar to normal control samples). The genes demonstrating at least a two-fold increase at peak in EAE tissue as opposed to non-involved tissue are set forth in Table 1, whereas genes having a decrease in expression in EAE tissue as opposed to non-involved tissue are set forth in Table 2. There are several genes which were previously known to be associated with EAE (e.g., which are differently

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expressed in MS tissue): TNF- α and IFN- β . The up-regulation of these genes served as validation to the method.

5 [0246] To generate the data in Tables 1 and 2, genes were clustered hierarchically into groups on the basis of similarity of their expression profiles by the procedure of Eisen et al. ((1998) Proc. Nat'l Acad. Sci. USA 95:25: 14863-8). Genes that were designated
10 absent (A) in all samples in a given experiment were eliminated from the analysis, as were -fold changes over the designated baseline of less than 2. Genes that were present (P) and were of unclear expression (M) were also indicated. Average fold changes between
15 peak/onset and peak/recovery stages were also calculated.

F. Identification of Immune Mediated Genes by
20 Comparison to *In Vitro* Expression Patterns

[0247] In addition, to identify disease-related, antigen-driven immune mediated genes, additional mice were immunized with an EAE-inducing factor and tissue
25 harvested for splenocytes. 3 SJL mice were immunized on Day 0 with 400 μ g pertussis toxin and 200 μ g injection of myelin oligodendrocyte glycoprotein (MOG) peptide residue 35-55 which, like PLP, induces EAE in mice. On Day 3, an additional dose of 400 μ g pertussis
30 toxin was administered. After 10 days, the spleens were harvested, and splenocyte cells were isolated and cultured *in vitro* with 5 μ g/ml MOG for 6 and 24 hours, to monitor expression of an immune response at an earlier time (6 hours) and at a later time (24 hours).
35 In addition, a subset of the splenocyte cells were

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further treated with 5 μ g/ml of anti-IL12p40 (C17.8) antibody, which is believed to produce inhibitory effects on the expression of MS (IL12p40 is an interleukin that induces IFN- γ expression in the Th-1 pathway).

[0248] Isolation of mRNA was performed as described above, with the *in vitro* samples being compared to murine 11,000 genes (Microarray, Affymetrix, Cat Nos. SubA #510243, SubB #510244). As shown in Table 3, MOG restimulation induced differential expression of 199 genes. Of these genes, Tables 4 and 5 indicate 17 genes which further responded to treatment with anti-IL12p40. In addition, quantitative analysis was performed by comparison of the *in vitro* samples to the *in vivo* studies, to identify common genes which were increased or decreased substantially, and/or which were affected by addition by MOG + antiIL12p40. Of the 1265 genes which were found to increase or decrease greater than three fold following MOG restimulation, 184 were found to be in common with genes expressed at peak disease of EAE. Further analysis revealed 6 genes in particular which also responded to treatment with anti-IL12p40 (shown in Table 5): CAPN12, MT1, MYO1F, TLN, UNK_AA117532 and UNK_AA645990.

EXAMPLE 2: IDENTIFICATION AND CHARACTERIZATION OF MARKER cDNA IN HUMAN MODEL OF MULTIPLE SCLEROSIS.

A. Study Design and Subject Entry Criteria

[0249] 60 patients diagnosed with varying stages of multiple sclerosis participated in the study from three sites: University Rochester (Rochester, NY), Lehigh Valley Hospital (Allentown, PA) and Institute of

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Neurology, University of London (London, UK). The University of Rochester and Lehigh Valley Hospital provided peripheral blood mononuclear cell (PBMC) samples, while the Institute of Neurology provided
5 brain lesion and non-lesional samples.

[0250] As is characteristic of MS, the patients manifested other neurological disorders and a wide panoply of symptoms, and were at different stages of
10 the disease: relapsing-remitting, 2° progressive, 1° progressive and acute exacerbation. The samples included post-mortem lesional and nonlesional brain samples, as well as other normal controls.

15 [0251] In general, the donor profile for PBMC samples had the following requirements: no current use of steroids (washout of 30 days), no current use of interferon or copaxone (washout 90 days), no previous history of cyclophosphamide use, no active viral
20 illnesses or infections and no current use of any other study drug.

B. Identification of MS markers from Blood or Brain

25 [0252] PBMC were isolated from blood samples using Lymphoprep (Nycomed, Oslo, Norway). Lesion and nonlesion brain samples were removed from brain stems and washed. To isolate infiltrating immune cells and microglial cells, the samples were run over percoll
30 gradient to remove neurons, endothelial cells and astrocytes.

[0253] As was performed in Experiment 1(C) above, RNA was isolated from the PBMC and brain samples using the

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RNeasy mini kit (Quiagen), the RNA probes were hybridized against human chip arrays having approximately 14,000 genes (MicroArray, Affymetrix, cat no. 510448) and quantitative analysis was performed using GeneChip. PBMC samples from MS patients were compared to PBMCs from undiseased patients, resulting in over 300 differentially regulated genes by more than 2 fold ($p < .01$), as shown in Table 7. Lesions from the MS brain were compared to nonlesion samples, resulting in over 100 genes, as shown in Table 8, that increased or decreased more than 2 fold ($p < .01$). A comparison of the genes expressed in brain and PBMC samples indicated that only 181 genes were shown to be present in both.

15 C. Comparison to *In Vitro* Expression Patterns

[0254] These genes were compared to genes identified in the murine samples of Example 1(F). Of the 181 genes which were either up- or down-regulated in the human PBMC and brain samples, 6 genes were shown to be in common with the murine genes that were differentially regulated *in vitro*: EEF1D, PIM2, PRDX2, SEC24C, UNK_AJ24 AND XIP (these genes and their accession numbers are listed in Table 9).

25

D. Taqman Polymerase Chain Reaction

[0255] To ensure that the data obtained from the GeneChip analysis (described above) was reflective of the actual level of gene expression in the cell samples, the expression of 4 selected cytokines, IL-10, IL-8, IL-12p35 and IL-1 β , were also measured by polymerase chain reaction, and the results compared to the expression levels of the same cDNAs of the cytokines in non-involved samples and in GeneChip

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analysis. Total RNA was treated with 10 units RQ1 Dnase I (Promega, Madison, WI) for 30 min at 37°C. Samples were extracted with phenol/chloroform, and RNA was precipitated with 0.3 M sodium acetate and 2
5 volumes of 100% ethanol. RNA was resuspended in DEPC-treated water, and the RNA concentration determined by measuring the optical absorbance at 260 nm. Then DNA polymerase was used to reverse transcribe and amplify
10 25 µg of total RNA in a single tube assay using the Perkin-Elmer TaqMan EZ RT-PCR kit (Perkin-Elmer Applied Biosystems, Foster City, CA) with gene-specific sense and anti-sense primers and a probe fluorescently labeled at the 5' end with 6-carboxyl-fluorescein (6-FAM) (Kruse (1997) J Immunol. Methods 210(2):195-203;
15 Heid et al. (1996) Genome Res 6(10):986-94). Primers and fluorescently labeled probes were generated using Primer Express software (Perkin-Elmer), and were synthesized by Perkin-Elmer. To avoid amplification of contaminating genomic DNA, primer pairs crossing
20 intron/exon boundaries were selected. Duplicate samples were reverse transcribed for 30 min at 60°C and then subjected to 40 rounds of amplification for 15 sec at 95°C and 1 min at 60°C using the ABI Prism 7700 sequence detection system as described by the
25 manufacturer (Perkin-Elmer) (Kruse et al. (1997) *supra*). Sequence-specific amplification was detected as an increased fluorescence signal of 6-FAM during the amplification cycle. Quantitation of gene-specific message levels was based on a comparison of the
30 fluorescence intensity in the unknown mRNA sample to the fluorescence intensity from the standard curve of known mRNA levels. Amplification of the gene for human acidic ribosomal protein (HARP) was performed on all samples tested to control for variations in RNA
35 amounts. MRNA of the above-mentioned genes were

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normalized to this control mRNA. The results are shown in Figure 1, and demonstrate that the average expression of the above-mentioned genes were increased in MS tissues relative non-involved tissues, as was
5 also confirmed from the GeneChip analysis.

E. MS CLASS PREDICTOR

[0256] A panel of differentially regulated genes were
10 selected and compiled for use in a class predictor model, by modifying the model described in Golub et al., "Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring" *Science*, 286: 531-537 (1999). The genes
15 were selected based on a "neighborhood analysis" of how well they correlated to a class distinction, e.g. MS-afflicted or not. Each class is represented by an idealized expression pattern of genes, in which the expression level is uniformly high in one class (e.g.
20 MS-afflicted) or uniformly low in the other (e.g. nondiseased). The panel of markers selected for the class predictor is shown in Table 10, together with their Accession numbers.

25 [0257] The prediction of whether a new sample is MS-afflicted or nondiseased is based on 'weighted votes' on the panel of selected genes. Each selected gene votes for either MS-afflicted or nondiseased, depending on whether its expression level X_i in the sample is
30 closer to μ_{MS} or $\mu_{non-diseased}$. The magnitude of the vote is $W_i V_i$, where W is a weighing factor that reflects how well the gene is correlated with the class distinction and $V_i = |X_i - (\mu_{MS} + \mu_{non-diseased})/2|$ reflects the deviation of the expression level in the sample from the average

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of μ_{MS} and $\mu_{non-diseased}$. The sample is assigned a qualitative outcome.

[0258] Validation of the class predictor was performed
5 by using samples from subjects known to be MS-afflicted
or nondiseased. As shown in Figure 2, 17 out of 18
samples (~94%) were correctly predicted as either MS-
afflicted or non-diseased, demonstrating that the class
predictor may be used as a viable means for diagnosing
10 a subject with multiple sclerosis. Additional data
points on the selected panel of genes should only bring
greater accuracy to the model's predictions. Moreover,
it is possible that with further compilation of data
from various stages or types of MS, the class predictor
15 may be able to different the MS disease stage of a
subject.

Other variations and modifications of this invention
will be obvious to those skilled in the art. This
20 invention is not limited except as set forth in the
claims.

NAME	Accession No.	Onset	Peak	Recovery	peak/onset	peak/recovery	feature
Immune related							
UNK_ET62206	ET62206	A 6	P 113	P 24	18.83	4.71	anti-digipatin immunoglobulin heavy chain variable region
IFRD1	v0756	A 5	P 22	A 4	4.40	5.50	interferon-related developmental regulator 1
IRG1	L39251	P 4	P 18	P 5	4.50	3.80	immunoresponsive gene 1
TNFAIP2	Msa.1583.0	P 7	P 19	P 5	2.71	3.80	tumor necrosis factor, alpha-induced protein 2
H2-BF	Msa.510.0	P 14	P 34	P 9	2.43	3.78	histocompatibility 2, complement component factor B
TGFBI	L19932	P 26	P 75	P 24	2.88	3.13	transforming growth factor, beta induced, 68 kDa
SCYA6	M56004	P 6	P 17	P 4	2.83	4.25	small inducible cytokine A6
IL1RN	m74294	P 11	P 24	P 12	2.19	2.00	interleukin 1 receptor antagonist
PBEF	aa691772	P 5	P 13	P 5	2.36	2.60	pre-B-cell colony-enhancing factor
SAA3	Msa.1160.0	P 20	P 40	A 2	2.00	20.00	serum amyloid A 3
SAA3	x03479	P 39	P 77	P 12	1.97	6.42	serum amyloid A 3
CS1B	U99827	P 6	P 24	P 8	4.00	3.00	cystatin B
IER3	X67644	P 11	P 31	P 11	2.93	2.82	immediate early response 3
cell structural							
TLN	x58123-2	M 4	P 16	A 3	4.00	5.33	talin
VIM	Msa.7648.0	A 9	P 39	P 17	4.33	2.28	vimentin
CAPG	X54511	P 11	P 23	P 11	2.12	2.08	capping protein (actin filament), gelsolin-like
MYO1F	Msa.3224.0	A 4	P 10	P 5	2.81	2.00	myosin II
metabolism/detoxification							
MT1	V00835	M 4	P 33	P 2	8.25	16.50	metallothionein 1
ADAM8	d10911	A 4	P 14	A 5	3.50	2.80	a disintegrin and metalloprotease domain 8
CNLP	y94353	A 7	P 47	A 12	6.71	3.92	cathelin-like protein
CH13L3	m94584	P 20	P 125	P 6	6.25	20.83	chitinase 3-like 3
ARG1	U51805	P 5	P 15	A 3	3.00	5.00	arginase 1, liver
ADFP	M93275	P 10	P 24	P 8	2.40	3.00	adipose differentiation related protein
GNA-RS1	Msa.2623.0	A 4	P 12	A 2	3.00	2.00	guanine nucleotide binding protein, related sequence 1 (GTP binding protein)
GNAI2	Msa.22597.0	P 11	P 24	P 12	2.32	2.00	guanine nucleotide binding protein, alpha inhibiting 2
LCN2	Msa.2129.0	A 4	P 11	A 4	3.00	2.75	lipocalin 2, assoc. with neutrophil gelatinase
Chromatin nuclear structure							
H3F3B	aa445408	P 3	P 17	P 8	5.67	2.13	H3 histone, family 3B
cell surface receptor/transporter							
CD37	aa274626	A 2	P 22	A 10	11.00	2.20	CD37 antigen-mostly on B cells
CD97	aa537407	A 3	P 10	P 5	3.22	2.00	CD97 antigen-leukocyte activation antigen
UNK_ET61559	ET61559	A 7	P 23	P 8	3.29	2.88	bradykinin B1 subtype receptor gene
LGALS3	Msa.3906.0	A 4	P 20	A 4	5.00	5.00	lectin, galactose binding, soluble 3
LGALS3	Msa.477.0	P 44	P 128	P 56	2.93	2.29	lectin, galactose binding, soluble 3
LTF	Msa.1271.0	P 7	P 15	P 5	2.14	3.00	lactoferrin
UNK_ET62839	ET62839	P 5	P 12	P 8	2.27	2.00	paired-immunoglobulin-like receptor-like receptor A1
UNK_L05630	Msa.491.0	A 5	P 10	A 5	2.05	2.00	G protein-coupled receptor (C5a)
protein processing/synthesis							
CAPN12	v64452	A 5	P 21	A 10	4.20	2.10	calpain 12
CASH	u97078	A 5	P 15	A 4	3.00	3.75	caspase homolog
EEF1A1	Msa.34019.0	A 7	P 18	A 4	2.57	4.50	eukaryotic translation elongation factor 1 alpha 1
LTF	Msa.1271.0	P 7	P 15	P 5	2.14	3.00	lactoferrin
SSR4	C78082	P 12	P 28	P 13	2.28	2.15	signal sequence receptor, delta
FABP5	Msa.2433.0	A 5	P 10	A 5	2.15	2.00	fatty acid binding protein 5, epidermal
cell cycle							
MAPK3	Msa.2276.0	P 7	P 29	P 13	4.14	2.23	mitogen activated protein kinase 3

Table 2

Name	Accession No.	Onset	Peak	Recovery	peak/on peak /recovery
Metabolism					
HMBS	Msa.1055.0	22	P	28	P 0.2727 0.2308 hydroxymethylbilane synthase
GNPI	w56954	18	P	18	P 0.3333 0.3333 glucosamine-6-phosphate deaminase
Protein processing					
ENPP2	Msa.20153.0	33	P	29	P 0.2727 0.3103 edonucleotide pyrophosphatase/phosphodiesterase 2
PPP3CB	Msa.531.0	18	P	19	P 0.2222 0.2105 protein phosphatase 3, catalytic subunit, beta isoform, brain
PPP2R1A	Z31244	17	P	10	P 0.1785 0.3 protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 85), alpha isoform
CAR2	k00811	53	P	41	P 0.34 0.439 carbonic anhydrase 2
ENPP2	Msa.13786.0	260	P	430	P 0.5192 0.314 edonucleotide pyrophosphatase/phosphodiesterase 2
IGFBP2	Msa.15111.0	33	P	20	P 0.2424 0.4 insulin-like growth factor binding protein 2
IGFBP6	X81584	24	P	18	P 0.2917 0.3889 insulin-like growth factor binding protein 6
Transcription					
ZFP35	x17617	15	P	7	P 0.2 0.4286 zinc finger protein 35
Cell division					
MCPR	X80169	18	P	21	P 0.3333 0.2857 meiotic check point regulator
Immunology regulated					
UNK_ET61953 ET61953		21	P	20	P 0.43 0.45 anti-DNA immunoglobulin light chain IgG
Oncogene					
MX2	J03368	21	P	20	P 0.48 0.5 myxovirus (influenza virus) resistance 2
ETS1	Msa.1440.0	31	P	38	P 0.45 0.3684 E28 avian leukemia oncogene 1, 5' domain
RGL1	U14103	16	P	14	P 0.25 0.2857 rat guanine nucleotide dissociation stimulator, like 1; binds ras p21
Transcription					
CUTL1	x75013	15	P	5	P 0.3333 0.4545 cut (Drosophila)-like 1
ESTs					
UNK_AA04862 aa048623		23	P	3	P 0.1304 0.4286 ESTs, Moderately similar to supported by EST T08756 [H.sapiens]
UNK_W74885 w74885		26	P	2	P 0.0769 0.069 ESTs, Weakly similar to 1-evidence
UNK_AA00014 aa000148		32	P	16	P 0.5 0.2182 ESTs, Highly similar to AF158168_1 pEachy [R.norvegicus]
UNK_W71423 w71423		13	P	2	P 0.1538 0.4 me40e04.r1 Soares mouse embryo N0MET3.5 14.5 Mus musculus cDNA clone 389982 5', mRNA sequence.
UNK_W91701 w91701		12	P	6	P 0.50 0.5 MTA.G08.089.A MTA adult mouse thymus library Mus musculus cDNA clone MTA.G08.089
UNK_AA00015 AA000151		18	P	7	P 0.39 0.4118 mg31b12.r1 Soares mouse embryo N0ME13.5 14.5 Mus musculus cDNA clone 425375 5'.
UNK_AA13783 aa137837		11	P	2	P 0.18 0.2222 mn01a03.r1 Soares mouse 3N0MS Mus musculus cDNA clone 598140 5'

Table 3

MOG induced at 6 and 24 Hours		MOG		6 hr MOG		24 hr MOG		24 hr MOG	
Name	Accession No.	avg WT	6 hr MOG	24 hr MOG	24 hr MOG	24 hr MOG	24 hr MOG	24 hr MOG	24 hr MOG
RBPSUH	X17459	8.5	20	39	1.95	1.85	1.85	1.85	1.85
UNK_AA170688	aa170688	34.5	123	228	1.85	1.85	1.85	1.85	1.85
MYC	100039	4.5	10	18	1.80	1.80	1.80	1.80	1.80
RAN	Msa.28092.0	10.5	29	52	1.79	1.79	1.79	1.79	1.79
D17WSU51E	w69907	7	17	30	1.78	1.78	1.78	1.78	1.78
SP18	U98700	6	16	28	1.75	1.75	1.75	1.75	1.75
FGL2	M16238	9	24	40	1.67	1.67	1.67	1.67	1.67
UNK_AA512178	aa512178	5	12	20	1.67	1.67	1.67	1.67	1.67
ICSBP	aa32489	23.5	57	93	1.63	1.63	1.63	1.63	1.63
UNK_AA273280	aa273280	9	27	44	1.63	1.63	1.63	1.63	1.63
UNK_Z31297	z31297	13	35	57	1.63	1.63	1.63	1.63	1.63
MINPP1	aa039115	8.5	16	26	1.63	1.63	1.63	1.63	1.63
HSP86-1	Msa.43197.0	22	57	89	1.56	1.56	1.56	1.56	1.56
D15WSU59E	Msa.6592.0	6	13	20	1.54	1.54	1.54	1.54	1.54
CATNS	Z17804	15	13	20	1.54	1.54	1.54	1.54	1.54
UNK_M28005	Msa.822.0	19	23	35	1.52	1.52	1.52	1.52	1.52
UNK_W34756	Msa.8882.0	5	10	15	1.50	1.50	1.50	1.50	1.50
UNK_AA063302	Msa.17538.0	9	14	21	1.50	1.50	1.50	1.50	1.50
SCYA4_Mm_AFFX	MIP1-B	16	64	95	1.45	1.45	1.45	1.45	1.45
UNK_AA103243	Msa.4225.0	6	21	31	1.45	1.45	1.45	1.45	1.45
XBP1	Msa.4597.0	10	28	38	1.46	1.46	1.46	1.46	1.46
DBWSU108E	Msa.3748.0	8	22	32	1.45	1.45	1.45	1.45	1.45
FPR1	Msa.106.0	40.5	82	119	1.45	1.45	1.45	1.45	1.45
D19WSU54E	Msa.5247.0	21	80	116	1.45	1.45	1.45	1.45	1.45
PSMB8	Msa.1629.0	66.5	184	264	1.43	1.43	1.43	1.43	1.43
L16	Msa.30325.0	68	187	265	1.42	1.42	1.42	1.42	1.42
UNK_W30651	Msa.4559.0	5.5	12	17	1.42	1.42	1.42	1.42	1.42
UNK_AA145180	Msa.39021.0	6.5	15	21	1.40	1.40	1.40	1.40	1.40
UNK_C78757	C78757	5	13	18	1.38	1.38	1.38	1.38	1.38
UNK_AA571638	aa571638	4.5	8	11	1.38	1.38	1.38	1.38	1.38
APOC1	Msa.13851.0	4.5	28	35	1.35	1.35	1.35	1.35	1.35
ITGB4BP	aa122622	5	12	16	1.33	1.33	1.33	1.33	1.33
APOC1	Msa.5982.0	6.5	42	56	1.33	1.33	1.33	1.33	1.33
CSF2	UNK_AA119959	4	9	12	1.33	1.33	1.33	1.33	1.33
CISH3	Msa.35530.0	12	33	44	1.33	1.33	1.33	1.33	1.33
CALM	U06328	8.5	35	46	1.31	1.31	1.31	1.31	1.31
RAN	Msa.18968.0	20.5	50	65	1.30	1.30	1.30	1.30	1.30
UNK_Z31269	Msa.39417.0	9	24	31	1.29	1.29	1.29	1.29	1.29
UNK_AA145971	Msa.39338.0	7.5	18	23	1.28	1.28	1.28	1.28	1.28
UNK_ET61864	ET61864	8.5	15	19	1.27	1.27	1.27	1.27	1.27
CSF2	X03019	15.5	42	53	1.26	1.26	1.26	1.26	1.26
FPR1	Msa.166.0	4	8	10	1.25	1.25	1.25	1.25	1.25
aa268897	aa268897	42.5	103	128	1.24	1.24	1.24	1.24	1.24
HSC70	Msa.22866.0	18	44	54	1.23	1.23	1.23	1.23	1.23
UNK_ET63083	ET63083	6	18	22	1.22	1.22	1.22	1.22	1.22
UNK_G12	Msa.6578.0	5	18	21	1.17	1.17	1.17	1.17	1.17
UNK_W62546	w62546	4.5	12	14	1.17	1.17	1.17	1.17	1.17
PSMB9	d14588	7	24	28	1.17	1.17	1.17	1.17	1.17
PSMB10	d85581	43	143	166	1.16	1.16	1.16	1.16	1.16
FCGR1	M31314	33	132	153	1.16	1.16	1.16	1.16	1.16
		7.5	19	22	1.16	1.16	1.16	1.16	1.16

recombining binding protein suppressor of hairless [Drosophila]
 ESTs, Weakly similar to tyrosine phosphatase [M.musculus]
 myelocytomatosis oncogene
 RAN, member RAS oncogene family
 DNA segment, Chr 17, Wayne State University 51, expressed
 serine protease inhibitor 6
 fibroblast-like protein 2
 ESTs, Highly similar to ARGINYL-TRNA SYNTHETASE [Chaeulius longicaudatus]
 interferon consensus sequence binding protein
 ESTs, Moderately similar to HYPOTHETICAL 58.5 KD PROTEIN T20812.3 IN CHROMOSOME III [Caenorhabditis elegans]
 ESTs, Weakly similar to AF154120.1 sorting nexin 1 [M.musculus]
 multiple inositol polyphosphate 1
 heat shock protein, 86 kDa 1
 DNA segment, Chr 15, Wayne State University 58, expressed
 calinin src
 Gag-env [provirus] [Mus musculus, MY, EV-2, murine AIDS virus-related provirus, Genomic Mutant, 3 genes, 4765 nt]
 ESTs, Moderately similar to URIDINE KINASE [Escherichia coli]
 Son cell proliferation protein
 SCY44 control sequence [M. musculus] [AFFX]
 nuclear RNA helicase Baf1
 X-box binding protein 1
 DNA segment, Chr 8, Wayne State University 108, expressed
 formyl peptide receptor 1
 DNA segment, Chr 18, Wayne State University 54, expressed
 proteasome (prosome, macropain) subunit, beta type 8 [large multifunctional protease 7]
 lymphocyte antigen 6 complex
 DNA segment, Chr 2, ERATO D01 303, expressed
 ESTs, Highly similar to TRANSLATIONAL INITIATION FACTOR 2 ALPHA SUBUNIT [Rattus norvegicus, Bos taurus]
 ESTs, Weakly similar to KIAA0841 protein [H.sapiens]
 vmd0a02.r1 Knowles Soller mouse blastocyst B1 Mus musculus cDNA clone 889542 5', mRNA sequence.
 apolipoprotein C1
 Integrin beta 4 binding protein
 apolipoprotein C1
 CSF2 control sequence [M. musculus] [AFFX]
 SEC23B (S. cerevisiae)
 cytokine inducible SH2-containing protein 3
 calmodulin
 RAN, member RAS oncogene family
 ESTs, Moderately similar to BIOTIN CARBOXYLASE [Anabaena pcc7120]
 ESTs, Highly similar to VACUOLAR ATP SYNTHASE SUBUNIT D [Bos taurus]
 Mus musculus FgamaRIB mRNA, complete cds.
 colony stimulating factor 2 (granulocyte-macrophage)
 formyl peptide receptor 1
 ESTs, Moderately similar to 7-80 [H.sapiens]
 heat shock protein cognate 70
 M.musculus PR284 gene.
 guanine nucleotide binding protein (G protein), gamma 12
 md03p05.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 373112 5', mRNA sequence.
 proteasome (prosome, macropain) subunit, beta type 9 [large multifunctional protease 2]
 proteasome (prosome, macropain) subunit, beta type 10
 Fc receptor, IgG, High affinity 1

Table 3

UNK_W4855	Msa.10700.0	9.5	26	30	1.15	mt24h11.1 Mus musculus cDNA, 5' end
UNK_AA181768	Msa.40979.0	6.5	13	15	1.15	DNA segment, Chr 6, ERATO Dot 748, expressed
UNK_AA110543	Msa.32918.0	5.5	14	16	1.14	ESTs, Highly similar to ARGINYL-TRNA SYNTHETASE [Citellus longicaudatus]
HSC70	Msa.33696.0	39.5	65	74	1.14	heat shock protein cognate 70
UNK_A4003590	Msa.16618.0	8	22	25	1.14	ESTs, Moderately similar to PRE-MRNA SPLICING FACTOR SRP20 [Homo sapiens; Mus musculus]
H2-123	Msa.25862.0	44	119	135	1.13	histocompatibility 2, T region locus 23
MAN2A1	X61172	14.5	30	34	1.13	mannosidase 2, alpha 1
SPI2-1	Msa.1178.0	31	187	204	1.09	serine protease inhibitor 2-1
UNK_W44201	Msa.10058.0	31	96	104	1.08	peptidylprolyl isomerase A
WARS	Msa.2538.0	5.5	48	52	1.08	pyrophosphoryl-ATP synthetase
TGTP	J38444	45.5	294	316	1.07	T-cell specific GTPase
NMI	AJ019249	20	85	89	1.08	N-myc (and STAT) interactor
UPP	D44484	16	112	118	1.05	uridine phosphorylase
ATP1B3	Msa.21575.0	7.5	19	20	1.05	ATPase, Na+/K+ beta 3 polypeptide
GBP2	aa288442	34.5	287	302	1.05	guanylate nucleotide binding protein 2
PPICAP	x87809	13.5	59	62	1.05	peptidylprolyl isomerase C-associated protein
CISH	z31843	8	25	28	1.04	cytokine inducible SH2-containing protein
TPST1	w61385	8.5	33	34	1.03	protein-tyrosine sulfotransferase 1
SCYA4_Mm_AFFX	MIP1-B	21.5	68	70	1.03	SCYA4 control sequence (M. musculus) [AFFX]
HSC70	Msa.7250.0	13.5	41	41	1.00	heat shock protein cognate 70
PSME1	Msa.41995.0	21.5	68	66	1.00	protease (prosome, macropain) 28 subunit, alpha
TNFRSF5	m83312	5.5	17	17	1.00	tumor necrosis factor receptor superfamily, member 5
UNK_AA072252	Msa.26801.0	4	13	13	1.00	phosphotransferase, platelet
JUN	Msa.4586.0	7	18	18	1.00	Jun oncogene
RPS5	Msa.29778.0	6	12	12	1.00	ribosomal protein S5
PSMB9	Msa.295.0	42	145	143	0.99	prosome (prosome, macropain) subunit, beta type 9 [large multifunctional protease 2]
UNK_AA013876	Msa.19552.0	48.5	94	92	0.98	mb08g10.1 Mus musculus cDNA, 5' end
IF203	a1022371	11.5	27	26	0.98	interferon activated gene 203
CCND2	m83749	5	24	23	0.96	cyclin D2
MG11	U15835	72.5	222	211	0.95	IFN-gamma induced
UNK_AA178518	aa178518	7	16	15	0.94	mt1402.1 Soares mouse 3NBMS Mus musculus cDNA clone 621027 5'
UNK_ET63461	ET63461	11.5	30	28	0.93	Mus musculus beta 1 integrin gene, partial cds.
IL3	x02732	6.5	15	14	0.93	interleukin 3
IGF1	Msa.4770.0	13.5	14	13	0.93	insulin-like growth factor 1
NIN1	aa169403	23.5	68	61	0.92	small inducible cytokine A1
SCYA1	m17957	4	12	11	0.92	small inducible cytokine A1
UNK_AA608277	AA608277	5	11	10	0.91	vn61g12.1 Baresad mouse proximal colon MPLRB8 Mus musculus cDNA clone 1025734 5' similar to gb:X68487 ADENOSINI
A02	J03368	11	18	16	0.89	myxovirus (influenza virus) resistance 2
IRF1	M21065	37	252	221	0.88	early development regulator 1
EDR2	aa682040	11	28	24	0.86	early development regulator 2 (homolog of polyhomeotic 2)
CDKN1A	w62380	9.5	42	38	0.86	cyclin-dependent kinase inhibitor 1A (P21)
UNK_AA137872	aa137872	16	41	35	0.85	ribosomal protein L35a
UNK_W59723	Msa.13968.0	9	61	52	0.85	ma38g08.r1 Mus musculus cDNA, 5' end
IFI204	m31419	8.5	27	23	0.85	interferon activated gene 204
ATPL	Msa.29770.0	19	54	48	0.85	ATPase-like vacuolar proton channel
IRF7	U73037	34	105	89	0.85	interferon regulatory factor 7
IGTP	U53219	33	185	156	0.84	interferon gamma induced GTPase
CCND2	Msa.25099.0	6	12	10	0.83	cyclin D2
IL1R2	x59769	11.5	38	30	0.83	interleukin 1 receptor, type II
IL2RG	Msa.136.0	28.5	79	65	0.82	interleukin 2 receptor, gamma chain
STAT1	Msa.289.0	9.5	68	54	0.82	signal transducer and activator of transcription 1
BZRP	Msa.32332.0	22	78	62	0.82	benzodiazepine receptor, peripheral
UNK_AA031158	Msa.22134.0	14.5	43	35	0.81	ESTs, Moderately similar to BIOTIN CARBOXYLASE [Anabaena pcc7120]
UNK_ET61871	ET61871	8.5	16	13	0.81	Mus musculus anti-DNA immunoglobulin heavy chain IgM mRNA, antibody 452p.18, partial cds.

TABLE 3

UNK_AA217487	69	180	146	0.81	ESTs, Weakly similar to myelin transcription factor 1-like [Mus musculus]
TAP2	8	42	34	0.81	ATP-binding cassette, sub-family B (MDR/TAP), member 3
IRG1	11.5	120	96	0.80	Immunoresponsive gene 1
UNK_C31621	6	15	12	0.80	ESTs, Weakly similar to T00389 hypothetical protein KIAA0615 - human [H. sapiens]
CASP11	8	37	29	0.78	caspace 11
SCYB9	4.5	118	89	0.75	small inducible cytokine B subfamily (Cys-X-Cys), member 9
UNK_W50837	11.5	44	33	0.75	ma22a05.r1 Mus musculus cDNA, 5' end
GBP3	16.5	118	87	0.75	guanylate nucleotide binding protein 3
PIM1	59	227	170	0.75	proliferation site 1
UNK_AA060167	15	102	76	0.75	pre-B-cell colony-enhancing factor
PNP	75.5	308	227	0.74	purine-nucleoside phosphorylase
ADORA2B	5	11	8	0.73	adenosine A2b receptor
UNK_Z31278	5	18	13	0.72	DNA segment, Chr 4, ERATO Dot 478, expressed
PPP2CA	5.5	14	10	0.71	protein phosphatase 2a, catalytic subunit, alpha isoform
PLAUR	14.5	35	25	0.71	urokinase plasminogen activator receptor
ITGB	7.5	17	12	0.71	integrin-associated protein
CDKN1A	7	20	14	0.70	cyclin-dependent kinase inhibitor 1A (P21)
IFI47	31.5	228	153	0.68	interferon gamma inducible protein, 47 kDa
ABCD1	4	9	6	0.67	ESTs, Moderately similar to INTERFERON-ACTIVATABLE PROTEIN 204 [Mus musculus]
BTG1	53	108	71	0.66	B-cell translocation gene 1, anti-proliferative
BCL3	5.5	22	14	0.64	B-cell leukemia/lymphoma 3
RGS1	5	11	7	0.64	regulator of G-protein signaling 1
EHO1	21.5	82	52	0.63	EH-domain containing 1
NFKBIA	80.5	192	120	0.63	nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha
WARS	4	8	5	0.63	tyllophary-RNA synthetase
ITGB	20.5	63	39	0.62	integrin-associated protein
FAS	5	13	8	0.62	tumor necrosis factor receptor superfamily, member 6
UNK_AA169469	11.5	65	40	0.62	ESTs, Weakly similar to Fin29 [H. sapiens]
UNK_AA146282	7.5	36	22	0.61	mi08e08.r1 Mus musculus cDNA, 5' end
UNK_M26005	5	10	6	0.60	Gag...env (provirus) [Mus musculus, MrV, Ew-2, murine AIDS virus-related provirus, Genomic Mutant, 3 genes, 4765 nt]
aa177851	10.5	32	19	0.59	mi01d08.r1 Soares mouse 3NBMS Mus musculus cDNA clone 619791 5'
GBP2	20.5	250	147	0.59	guanylate nucleotide binding protein 2
UNK_AA153032	25	114	67	0.59	ESTs, Highly similar to INTERFERON-ACTIVATABLE PROTEIN 204 [Mus musculus]
IFI203	11	53	31	0.58	interferon activated gene 203
UNK_AA082397	5	12	7	0.58	ESTs, Highly similar to RAS-RELATED PROTEIN RAB-5A [Canis familiaris]
ACTC1	8.5	33	19	0.58	actin, alpha, cardiac
IL15RA	6.5	14	8	0.57	interleukin 15 receptor, alpha chain
IDB1	6	21	12	0.57	inhibitor of DNA binding 1
LGALS9	25.5	72	41	0.57	lectin, galactose binding, soluble 9
UNK_AA152988	4.5	16	9	0.56	ESTs, Highly similar to RAS-RELATED PROTEIN RAC1 [Caenorhabditis elegans]
MAPKAPK2	9.5	29	16	0.55	MAP kinase-activated protein kinase 2
IQGAP1	17	44	24	0.55	DNA segment, Chr 7, ERATO Dot 237, expressed
UNK_AA289585	5.5	13	7	0.54	Mus musculus clone L5 uniform group of 2-cell-stage gene family mRNA, complete cds
UNK_ET62934	7	15	8	0.53	M. musculus antibody heavy chain variable region (348bp).
H2-K	28.5	82	33	0.53	Histocompatibility 2, K region
UNK_AA153196	15.5	38	20	0.53	Mus musculus Ube11 mRNA, partial cds
IFI204	8.5	38	20	0.53	interferon activated gene 204
NFKBIA	6	14	7	0.50	nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha
UNK_AA125310	6	14	7	0.50	ESTs, Highly similar to LEUKOCYTE ELASTASE INHIBITOR [Homo sapiens]
FAS	7	12	6	0.50	FAS control sequence (M. musculus) [AFEX]
UNK_ET62870	10	14	7	0.50	Mus musculus immunoglobulin heavy chain variable region (Igh) mRNA, partial cds.
D10WSU93E	8.5	53	28	0.49	DNA segment, Chr 10, Wayne State University 83, expressed
MYD88	26	98	47	0.49	myeloid differentiation primary response gene 88
IL1B					Interleukin 1 beta

Table 3

UNK_A4013783	Msa.5720.0	8.5	45	22	0.49	ESTs, Highly similar to INTERFERON-INDUCED 35 KD PROTEIN [Homo sapiens]
DAXX	AF06040	26	68	33	0.49	Fas death domain-associated protein
CASP8	Msa.28381.0	5	17	8	0.47	caspase 8
FAS	FAS	6	17	8	0.47	FAS control sequence (M. musculus) [AFFX]
UNK_ET61797	ET61797	6	13	6	0.46	Mus musculus anti-DNA immunoglobulin heavy chain IgG mRNA, antibody 363s.62, partial cds.
UNK_X52622	Msa.2275.0	4	9	4	0.44	Mouse IN gene for the integrase of an endogenous retrovirus
UNK_ET62928	ET62928	9	16	7	0.44	Mus musculus antibody heavy chain variable region (366bp).
HOC	X57437	14.5	55	24	0.44	histidine decarboxylase cluster
ISG15	X56602	83	381	163	0.43	interferon-stimulated protein (15 kDa)
UNK_AA163967	Msa.41726.0	4.5	12	5	0.42	mi24g07.1 Mus musculus cDNA, 5' end
UNK_A4072573	Msa.28697.0	5.5	46	19	0.41	pre-B-cell colony-enhancing factor
BACTIN5_ST_Mm	AFFX-b-ActinMm1	7.5	17	7	0.41	Beta-actin 5' control sequence (M. musculus) [AFFX]
TNF	X02811	8	42	17	0.40	tumor necrosis factor
CDKN1A	Msa.1362.0	7.5	23	9	0.39	cyclin-dependent kinase inhibitor 1A (P21)
UBL1A2.PENDING	Msa.20430.0	10.5	26	10	0.38	ubiquitin-like 1 (sentrin) activating enzyme subunit 2
IFIT2	U43085	15.5	94	36	0.38	Interferon-induced protein with tetratricopeptide repeats 2
UNK_L32973	L32973	33	130	48	0.37	thymidylate kinase homologue
USP18	a226391	21.5	106	39	0.37	ubiquitin specific protease 18
BACTIN5_ST_Mm	AFFX-b-ActinMm1	6.5	14	5	0.36	Beta-actin 5' control sequence (M. musculus) [AFFX]
ACTX	Msa.32804.0	47	174	62	0.36	melanoma X-actin
SCYA4	m35590	4.5	17	6	0.35	small inducible cytokine A4
L-CCR	Msa.22422.0	13	111	37	0.33	lipopolysaccharide inducible C-C chemokine receptor related
RGS1	Msa.37566.0	5	12	4	0.33	regulator of G-protein signaling 1
MAPK13	Msa.8386.0	5	12	4	0.33	mitogen activated protein kinase 13
SCYB10	m33268	14	265	78	0.29	small inducible cytokine B subfamily (Cys-X-Cys), member 10
TNF	TNFA	5	24	7	0.29	TNF control sequence (M. musculus) [AFFX]
IFIT3	I32874	43.5	182	53	0.29	interferon-induced protein with tetratricopeptide repeats 3
MX1	m12279	7	36	10	0.28	myxovirus (influenza virus) resistance 1
ICAM1	X52264	5.5	44	12	0.27	intercellular adhesion molecule
TNF	TNFA	4.5	30	8	0.27	TNF control sequence (M. musculus) [AFFX]
UNK_ET61450	ET61450	4	15	4	0.27	Mus musculus interleukin-2 (IL-2) mRNA, strain-specific RF allele, partial cds.
IFIT1	U43084	27	131	32	0.24	IL2 control sequence (M. musculus) [AFFX]
UNK_X73040	IL2	4	19	4	0.21	interferon-induced protein with tetratricopeptide repeats 1
L-CCR	AF030185	5	24	5	0.21	lipopolysaccharide inducible C-C chemokine receptor related
UNK_ET63426	ET63426	5	29	6	0.21	Mouse strain NOD tumor necrosis factor alpha (TNFA) gene, exon 2 and 3.
IFIT1	a616578	35.5	179	37	0.21	interferon-induced protein with tetratricopeptide repeats 1
UNK_X73040	IL2	4	23	4	0.17	IL2 control sequence (M. musculus) [AFFX]
MAD	X83106	9	78	9	0.12	Max dimerization protein
MAPKAPK2	Msa.2435.0	8.5	51	5	0.10	MAP kinase-activated protein kinase 2
UNK_D19407	D19407	4	42	4	0.10	MUSGS00778 Mouse 3'-directed cDNA; MUSGS00778; clone mb0657.

Table 4

MOG Induced and Blocked by anti-IL12				
Name	Accession No.	Baseline	MOG	WT+MOG+antiIL12p40
D2WSU58E	aa409818	9	7	5
TCFL1	Msa.6010.0	12	17	14
BPGM	X13586	75	62	82
UNK_W10325	w10325	10	7	9
MRJ-PENDING	aa499641	12	12	13
ANXA4	Msa.10010.0	4	6	5
TAF2E	D49439	5	8	4
UNK_AA408555	aa408555	9	6	4
SCD2	w41032	5	6	6
UBCE7	w78245	16	17	13
UNK_AA254104	aa254104	15	4	5
UNK_ET61871	ET61871	7	16	5
UNK_AA472320	aa472320	17	16	15
UNK_AA213318	aa213318	11	11	11
UNK_ET61737	ET61737	20	21	22
UNK_AA198324	AA198324	13	17	13
SDF2	D50646	17	10	11

Table 5

Accession No.	Name	Baseline	MOG	WT	MOG+antiL12p40	
w84452	CAPN12	5	11	5		calpain 12
V00835	MT1	4	12	4		metallothionein 1
Msa_3234.0	MYO1F	5	16	8		myosin II
x56123-2	TLN	4	16	8		talin
aa045990	UNK_AA045990	4	19	7		ESTs, Highly similar to GLUTAMINYL-TRNA SYNTHETASE (Homo sapiens)
aa117532	UNK_AA117532	7	41	16		ESTs, Moderately similar to HYPOTHETICAL 16.7 KD PROTEIN MRP17-MET14 INTERGENIC REGION (Saccharomyces cerev

Table 6

	A	B	C	D	E
1	Table of Genes Known to be Associated with MS				
2					
3	IL-12p40				
4	IL-10				
5	ICAM-2				
6	GAP-43				
7	a-2 chimmaerin				
8	myelin-transcription factor-1				
9	activin type II receptor				
10	retinoic acid receptor α -1				
11	EF-1				
12	neuron-specific enolase				
13	proton ATPase				
14	Glutamyl-tRNA synthetase				
15	Na, K-ATPase beta subunit				
16	neuroendocrine-specific protein A				
17	alpha-N-acetylgalactosaminidase				
18	liver type alkaline phosphatase				
19	male-enhanced antigen				
20	mitochondrial enoyl-CoA hydratase				
21	import precursor-ATP synthase α				
22	serine dehydratase				
23	adenine nucleotide translocator-2				
24	ornithine decarboxylase antienzyme				
25	protein kinase C type β -1				
26	cell surface glycoprotein A15				
27	IRF-2				
28	56-K autoantigen annexin XI				
29	high endothelial venule				
30	leukotriene A-4 hydrolase				
31	transcription factor E2A				
32	TNF α receptor-2				
33	duffy chemokine receptor				
34	MS-1				
35	PRG-2				
36	MBP				
37	protein tyrosine phosphatase, receptor type, C				
38	MOG				
39	Interferon β 1				
40	ICAM-1				
41	CTLA4				
42	APOE				

Table 7

Gene Name	Accession #	MS Avg	MS SD	Norm Avg	Norm SD	p value	FC MS/MS Description
XAP1	U87222	52.20	18.65	3.77	3.03	0.0039	14.11 H9V associated factor
OUA18	A006250	52.60	18.97	6.46	4.82	0.0090	6.14 acid-inhibitable phosphoprotein
KIA0313	A0062311	13.00	5.34	2.03	1.38	0.0095	6.28 POZ domain containing guanine nucleotide exchange factor(GEF)-1; RA(Ras/Rap1A-associating-GEF
PPF3CC	S46022	68.60	14.36	12.00	7.72	0.0004	5.72 protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform (calcineurin A gamma)
KIA00742	A0016285	81.80	18.89	15.54	8.07	0.0010	5.26 KIA00742 protein
UNC1A02238	A0022398	21.00	5.05	5.85	4.19	0.0009	3.59 Cluster 1 and A022398; Homo sapiens DNA sequence from PAC 434014 on chromosome 1q32.3-41. Contains the HSD11B1 gene for Hydroxysteroid (11-beta
LSPF2	R56605	18.00	5.52	5.54	5.06	0.0027	3.43 linked to Surfeit genes in Fugu subspes 2; LSPF2 gene 2
UNC1A07076	A0070768	18.00	5.72	5.85	4.54	0.0038	3.39 Homo sapiens cDNA FLJ13967 fls, clone Y79AA1001402, weakly similar to Homo sapiens paraneoplastic cancer-testis-brain antigen (MA4) mRNA
TBCA	U81224	16.20	4.87	4.85	3.51	0.0038	3.34 tubulin-specific chaperone c
MEC	A014837	24.80	9.07	7.46	8.89	0.0078	3.32 putative methyltransferase
BCR	X02596	18.00	4.69	5.85	4.79	0.0015	3.08 breakpoint cluster region
RAB27A	U07094	32.40	4.83	10.54	6.85	0.0000	3.07 RAB27A, member RAS oncogene family
10TF6	AF040704	13.40	4.22	4.38	3.12	0.0053	3.06 putative tumor suppressor
PAP	X76770	20.40	6.62	6.77	9.36	0.0057	3.01 poly(A) polymerase
HNRPH3	AF052131	23.80	4.97	7.92	6.37	0.0003	3.00 heterogeneous nuclear ribonucleoprotein H3 (H9)
DNF1	A1656421	32.80	6.19	10.92	8.99	0.0001	2.88 cyclin D-binding Myb-like protein
LOC56270	AF091083	14.00	2.92	4.69	2.36	0.0008	2.98 hypothetical protein 628
ARCN1	X81198	12.40	2.86	4.23	3.06	0.0008	2.93 archanin 1
OGT	U77413	16.40	3.44	5.62	3.28	0.0005	2.92 O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)
GLS	A0020645	74.20	13.83	25.62	16.07	0.0002	2.90 glutaminase
PRKACB	N34181	17.60	4.56	6.08	5.50	0.0015	2.90 protein kinase, cAMP-dependent, catalytic, beta
PTN22	AF001648	112.80	15.01	39.00	25.96	0.0000	2.89 protein tyrosine phosphatase, non-receptor type 22 (tyrosinoid)
TRAF5	A000509	52.00	14.00	18.00	8.84	0.0001	2.88 TNF receptor-associated factor 5
D6F72P64004	A0050390	65.00	11.25	22.62	12.93	0.0001	2.87 hypothetical protein DNF25640043
KIA0037	D07244	11.00	2.83	3.85	1.88	0.0025	2.86 KIA00247 gene product
FLTLG	U03558	36.20	7.76	12.69	12.25	0.0004	2.85 lms-related tyrosine kinase 3 ligand
APBA3	A0021638	48.40	11.41	17.00	9.84	0.0013	2.85 amyloid beta (A4) precursor protein-binding, family A, member 3 (X11-like 2)
KIA00603	A0011175	16.20	5.17	5.69	3.45	0.0071	2.85 KIA00603 gene product
KIA00855	A0020682	10.80	4.21	6.62	4.07	0.0008	2.84 poligin-87
KIA00540	A0011112	50.00	12.31	17.62	13.91	0.0012	2.84 KIA00540 protein
HIVP2	A0023584	12.00	2.45	4.23	2.85	0.0004	2.84 human immunodeficiency virus type 1 enhancer-binding protein 2
SUC111	D80000	27.80	7.40	9.85	8.82	0.0020	2.82 SNCT1 (structural maintenance of chromosomes 1, yeast)-like 1
SUL1A2	U14804	11.20	2.28	4.00	2.16	0.0005	2.80 sulfotransferase family, cytosolic, 1A, phenol-preferring, member 2
KIA00542	A0011114	11.60	3.05	4.15	2.97	0.0022	2.79 KIA00542 gene product
KIA00907	A0020714	26.60	7.96	9.54	7.48	0.0045	2.79 KIA00907 protein
IKBKG	AF031416	21.20	6.72	7.62	5.59	0.0084	2.78 inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
POLR2H	U37689	21.40	6.95	7.69	5.08	0.0077	2.78 polymerase (RNA) II (DNA directed) polypeptide H
MYO47	AF063605	28.80	7.19	10.38	6.45	0.0018	2.77 MYO47 protein
RNF4	A0000468	61.40	14.33	22.15	12.85	0.0012	2.77 rho finger protein 4
NKX2	AF043325	13.20	4.21	4.77	2.92	0.0074	2.77 Nucleosyltransferase 2
FBXO21	A0020682	10.00	3.08	3.62	1.89	0.0069	2.77 F-box only protein 21
DDX1	X70649	20.40	5.73	7.38	6.02	0.0031	2.76 DEAD-box (Arg-Glu-Ala-Asp-His) box polypeptide 1
RSP1	N04673	15.00	3.08	5.48	3.71	0.0004	2.75 Heat shock transcription factor 1
SIP	AF080581	12.20	4.02	4.46	2.40	0.0095	2.73 SYT interacting protein
ADP3	A0013325	13.40	2.41	4.92	3.50	0.0001	2.72 aquaporin 3
MSH42	K27384	15.00	4.47	5.54	3.99	0.0049	2.71 membrane-spanning 4-domains, subfamily A, member 2
EZH1	A0002386	20.80	4.39	7.62	5.66	0.0005	2.71 enhancer of zeste (Drosophila) homolog 1
RAB2L	AJ050259	17.00	1.41	6.31	5.31	0.0000	2.70 RAB2, member RAS oncogene family-like
GRB2	M86995	11.60	1.52	4.31	1.93	0.0000	2.69 growth factor receptor-bound protein 2
PSCD1	M85169	31.20	9.73	11.62	8.33	0.0084	2.69 pleckstrin homology, Sec7 and coiled-coil domain 1 (cytohesin 1)
BTGAL3	U09548	18.80	4.82	7.00	6.77	0.0019	2.69 bulkyophlin, subfamily 3, member A3
MPV17	X75538	22.00	3.54	8.23	6.76	0.0001	2.67 MPV17 transgene, murine homolog, glomerulosclerosis
TGFBR2	D06883	91.60	28.10	34.31	25.85	0.0038	2.67 transforming growth factor, beta receptor II (TGF-betaR)
RAF1	X03484	23.20	4.32	8.69	5.79	0.0002	2.67 v-src-1 murine leukemia viral oncogene homolog 1
KIA00129	D50919	15.60	4.39	5.85	5.89	0.0035	2.67 KIA00129 gene product
RBM6	AF089517	24.20	7.29	9.08	6.17	0.0056	2.67 RNA binding motif protein 6
TNRC1	U06760	16.20	3.27	6.08	4.01	0.0004	2.67 intracellular repeat containing 1
C10RF4	AL080097	55.20	8.59	20.77	11.60	0.0000	2.68 chromosome 3 open reading frame 4
ADH5	M81118	11.00	3.24	4.15	2.30	0.0057	2.65 alcohol dehydrogenase 5 (class III), ch polypeptide
UNK_A044279	A0442799	22.40	7.47	8.46	5.80	0.0037	2.65 Cluster 1 and A442799; x65910.11. Scarpia, total. beta. Homo sapiens cDNA clone IMAGE:588755. mRNA sequence.
NUP200	A1131168	23.80	4.38	9.00	6.24	0.0002	2.64 Nuclear matrix protein NUP200 related to nucleic acid factor PRP19
LANC1	A1131395	75.40	16.44	28.54	21.94	0.0006	2.64 LncC (lactical linoleic synthase component C)-like 1
UNK1	A0005688	39.00	6.03	14.77	8.09	0.0008	2.64 signal-induced proliferation-associated gene 1
SIPK1	A0022312	13.00	2.45	4.82	4.05	0.0002	2.64 Cluster 1 and A022312; Human DNA sequence from clone RPS-104E15 on chromosome 22q12.3-13.1 Contains the MGA73 gene for mannosyl (beta-1,4)-gly
RBM4	U95905	13.20	3.70	5.00	4.47	0.0034	2.64 RNA binding motif protein 4
TUBG3	X07103	13.80	3.13	5.15	3.85	0.0010	2.64 Tubulin, alpha, brain-specific
P130	D21262	16.40	5.03	6.23	4.57	0.0061	2.63 nuclear phosphoprotein p130

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RASGE2	A168812	35.00	3.54	13.69	9.99	0.0000	2.63 RAS guanylate releasing protein 2 (calcium and DAG-regulated)
KIA00746	A8018289	59.20	19.56	22.54	16.44	0.0060	2.63 KIA00746 protein
HIF-1A	U224311	22.00	6.80	6.28	9.33	0.0058	2.62 hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
KIA00986	A8020713	47.80	6.93	16.23	13.96	0.0000	2.62 KIA00986 protein
RAC1	U28870	80.80	8.70	30.85	18.76	0.0000	2.62 ras-related G binding protein subunit 1 (no family, small GTP binding protein Rac1)
RFC4	U50682	13.20	3.42	5.65	4.34	0.0009	2.60 interferon regulatory factor 4
MTA1	U33113	11.80	3.63	4.54	3.18	0.0007	2.60 metastasis associated 1
PHF1	AFO42386	12.20	2.17	4.69	3.20	0.0001	2.60 peptidylprolyl isomerase E (cyclophilin E)
THRC11	AFO17309	22.80	5.40	8.77	7.14	0.0012	2.60 nucleotide repair containing 11 (THR-associated protein, 230 kDa subunit)
YME1L1	A1326317	60.60	15.71	23.54	22.04	0.0024	2.57 YME1 (S. cerevisiae) Mte 1
MCM6	D04557	39.80	7.29	15.46	14.32	0.0003	2.57 minichromosome maintenance deficient (mss. S. pombe) 6
ZNF81	L11672	19.60	6.02	7.82	5.82	0.0055	2.57 zinc finger protein 81 (HPF7, HTP-10)
PTD017	A4152202	35.60	10.01	13.85	9.49	0.0041	2.57 PTD017 protein
KIA00982	A8023199	68.40	21.28	26.82	17.02	0.0075	2.57 KIA00982 protein
KIA00989	A8020716	87.80	10.78	34.23	26.47	0.0000	2.56 small nuclear ribonucleoprotein polypeptide A
SNRPA	M60784	72.20	9.39	28.23	21.55	0.0000	2.55 Cluster and AC004331: Homo sapiens Chromosome 16 BAC clone C1797SK-44M2, complete sequence.
UNK_A000438	AC004381	10.80	3.27	4.23	2.98	0.0063	2.55 ATPase, H+ transporting, lysosomal (vacuolar proton pump), alpha polypeptide, 70kD, isoform 1
ATP6A1	A4056747	73.20	17.81	28.69	17.72	0.0019	2.53 KIA00922 protein
KIA00922	A8023139	13.60	3.85	5.38	3.12	0.0050	2.52 heterogeneous nuclear ribonucleoprotein R
HNRPR	AFO00364	40.00	9.81	15.85	11.22	0.0000	2.52 integral membrane protein 2A
ITGA2A	A021786	12.80	16.92	31.54	24.35	0.0007	2.52 transcription factor 3 (E2A immunoglobulin enhancer binding factors E12E47)
TCF3	M15123	79.40	16.92	31.54	24.35	0.0007	2.52 son of sevenless (Drosophila) homolog 1
SOS1	A6027802	41.20	11.29	16.38	12.30	0.0038	2.51 amino-terminal enhancer of split
IGKC	M63436	11.80	3.21	4.62	3.20	0.0040	2.51 immunoglobulin kappa constant
KIA00999	D439511	23.00	3.61	9.15	7.27	0.0001	2.51 KIA00999 gene product
HTD10	AL049946	13.80	3.27	6.31	4.17	0.0008	2.50 uncharacterized hypothetical protein HTD10
CASP	U31346	10.20	2.39	4.08	2.96	0.0019	2.50 calpastatin
DSR1E	Z37166	10.90	2.35	4.00	3.81	0.0025	2.50 interferon 2 receptor, gamma (severe combined immunodeficiency)
IL2RG	D11086	14.80	3.63	5.92	4.41	0.0018	2.50 DNF2P34D156 protein
TYRO2	X54637	14.80	2.28	5.92	3.71	0.0000	2.50 tyrosine kinase 2
ATP2A3	Y15724	32.40	6.47	7.77	7.54	0.0008	2.50 dual specificity phosphatase 11 (PNAFNP complex 1-interacting)
ATR	U48844	13.60	3.13	5.48	4.46	0.0013	2.49 ATRase, Ce++ transporting, ubiquitous
UNK_AFO5499	AFO54996	26.80	3.19	10.77	6.98	0.0000	2.49 ataxia telangiectasia and Rad3 related
GOSR1	AFO47438	13.00	2.92	5.23	3.42	0.0011	2.49 Homo sapiens clone Z3763 mRNA sequence
CPSF1	U37012	50.00	6.44	20.15	13.92	0.0000	2.49 golgi SNAP receptor complex member 1
DDX19	AZ27946	20.20	4.09	8.15	6.88	0.0008	2.48 DEAD/H (Asp-Glu-Ala-Asp/Glu) box polypeptide 19 (Ddp5, yeast, homolog)
WSSCR1	D26088	16.00	4.30	6.46	4.58	0.0035	2.48 Williams-Beuren syndrome chromosome region 1
KIA00164	D75986	23.80	6.30	9.82	7.82	0.0031	2.48 KIA00164 gene product
UNK_A1748192	A1748193	27.20	5.63	11.00	9.57	0.0007	2.47 Homo sapiens mRNA: cDNA DKFZ586K123 (from clone DKFZ586K123)
SIT	U310659	76.60	14.31	31.00	18.03	0.0004	2.47 SHP2 interacting transmembrane adaptor
KIA00988	A8023216	26.20	8.44	10.82	7.04	0.0067	2.47 KIA00988 protein
EWSR1	X68595	14.60	4.34	5.92	4.03	0.0063	2.46 Ewing sarcoma breakpoint region 1
BRD1	AB018356	29.20	3.22	11.55	8.74	0.0002	2.46 alkylation-resistant 9 (CUP-Neurospora crassa) alpha-2,3-sialyltransferase: GM3 synthase
JUND	X56681	10.40	1.52	4.23	2.77	0.0000	2.46 jun D proto-oncogene
UNK_A03128	AL031282	20.80	2.17	8.46	6.60	0.0000	2.46 Cluster and AC031282: Human DNA sequence from clone 293E3 on chromosome 1p38.21-38.33. Contains the alternatively spliced gene for Matrix Metallopro-
PURA	M95684	10.20	2.77	4.15	2.34	0.0044	2.46 RNA binding protein; AT-rich element binding factor
SRM000	A8002322	17.00	5.24	6.92	4.31	0.0081	2.45 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4
SMARCA4	U79175	13.40	3.65	5.46	5.55	0.0044	2.45 RNA binding protein; AT-rich element binding factor
DATF1	A8002331	10.00	3.00	4.08	1.98	0.0081	2.45 SMN2 associated transcription factor 1
ARHGFE2	U72206	33.20	3.03	13.54	11.01	0.0000	2.45 nuclear phosphatase 2, regulatory subunit B (B56), gamma isoform
PP2R5C	Z69030	13.20	3.96	5.38	3.73	0.0088	2.45 protein phosphatase 2, regulatory subunit B (B56), gamma isoform
NCF1	M55067	22.40	3.21	9.15	5.86	0.0000	2.45 neurophil cytosolic factor 1 (NCF1), chronic granulomatous disease, autosomal 1
UBA52	AC005253	22.00	5.15	9.00	6.35	0.0015	2.44 ubiquitin A-52 residue ribosomal protein fusion product 1
OMP2	AL050350	57.60	13.87	23.62	16.57	0.0018	2.44 Opa-interacting protein 2
PPP2R2A	M64629	24.00	2.92	9.95	6.45	0.0000	2.44 protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform
NUMA1	Z11584	11.80	2.17	4.85	3.26	0.0003	2.43 nuclear mitotic apparatus protein 1
SSNA1	Z98932	20.60	3.51	8.46	5.71	0.0001	2.43 Spoptin3 syndrome nuclear autoantigen 1
KIA01086	A196457	34.80	1.30	14.31	9.69	0.0000	2.43 KIA01086 protein
EFSA	U17689	11.40	1.52	4.68	3.28	0.0000	2.43 eukaryotic translation initiation factor 5A
TTCC3	D94077	37.00	9.30	15.23	10.60	0.0039	2.43 tetrapeptide repeat domain 3
TTG2	Z75331	14.20	2.66	5.85	5.13	0.0007	2.43 stromal antigen 2
CTC13	L10910	19.80	3.70	8.15	7.05	0.0005	2.43 stromal antigen 2
MYC	V00568	18.80	3.27	8.15	6.50	0.0002	2.43 v-myc avian myelocytomatous viral oncogene homolog
FLJ20479	A016004	25.40	8.20	10.46	9.25	0.0098	2.43 likely homolog of yeast Hsp26, component of the Hsp70 chaperone
SLC25A1	X96924	24.60	2.17	10.23	5.76	0.0000	2.42 acute cancer family 25 (mitochondrial carrier, citrate transporter), member 1

Table 7

UBE1C	AF046024	11.00	1.59	4.54	2.87	0.0000	2.42 ubiquitin-activating enzyme E1C (homologous to yeast UBA3)
USP2	U27160	11.00	3.39	4.54	2.30	0.0093	2.42 UDP-glucose pyrophosphorylase 2
HRP40	U23803	21.60	5.02	9.00	6.71	0.0043	2.42 heterogeneous nuclear ribonucleoprotein A0
CBF2	U23803	15.00	3.24	6.82	4.27	0.0008	2.42 CCAAT-box-binding transcription factor
RHM5	U23840	58.60	13.52	24.25	16.28	0.0015	2.42 RNA binding motif protein 5
CHIL	AF104398	21.20	4.92	6.77	5.81	0.0016	2.42 comethion-like
LOC51669	W26859	10.60	1.14	4.39	2.99	0.0000	2.42 HSPC35 protein
KU40801	A018244	63.00	9.60	26.08	16.96	0.0000	2.42 KU40801 gene product
SCA2	Y08262	10.40	2.97	4.31	3.01	0.0054	2.41 spinocerebellar ataxia 2 (dihydropyrimidinase-like 2)
UNK_AL04947	AL049471	10.40	2.70	4.31	3.01	0.0043	2.41 Homo sapiens mRNA; cDNA DKFZ568N012 (from clone DKFZ568N012)
EIF4G1	U12688	20.20	3.90	8.38	6.90	0.0005	2.41 eukaryotic translation initiation factor 4 gamma, 1
POLG	U65025	10.00	2.12	4.15	2.08	0.0011	2.41 polymerase (DNA directed), gamma
GADD45B	AF078077	12.40	2.88	5.15	3.93	0.0016	2.41 growth arrest and DNA-damage-inducible, beta
UNK_X56199	X56199	14.90	2.68	6.15	4.62	0.0003	2.41 Human XIST, coding sequences a mRNA (locus DXS398E)
CSF3R	M59818	12.20	2.49	5.08	3.30	0.0006	2.40 colony stimulating factor 3 receptor (granulocyte)
SLC9A3R1	AF015928	41.00	10.42	17.08	11.42	0.0028	2.40 solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1
DKFZP56022	AL080214	16.40	2.07	6.85	4.41	0.0000	2.39 ATP-binding cassette, sub-family C (GFTABP), member 10
ARCC10	A004207	11.60	2.51	4.85	3.00	0.0010	2.39 Homo sapiens mRNA; cDNA DKFZ568N123 (from clone DKFZ568N123)
UNK_AL08021	AL080218	13.80	3.27	5.77	4.53	0.0018	2.39 ubiquitin-conjugating enzyme E2G 2 (homologous to yeast UBC7)
UBE2G2	AF02456	74.60	17.10	31.25	24.26	0.0015	2.39 periodosome biogenesis factor 10
KU40546	A011118	18.00	4.85	7.54	5.16	0.0041	2.38 ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)
PEX10	A019459	13.40	2.86	5.62	4.31	0.0010	2.38 SH3 domain-containing protein 6511
ADPRT	J03473	17.60	4.83	7.38	6.42	0.0046	2.38 heterogeneous nuclear ribonucleoprotein D-like
LOC51165	W27824	19.60	3.91	8.23	5.07	0.0006	2.38 hypothetical protein
HNRPOL	D99878	23.60	3.05	9.92	7.08	0.0000	2.38 RAD23 (S. cerevisiae) homolog A
DKFZP566713	AL050073	27.60	7.54	11.62	6.10	0.0044	2.38 protein phosphatase 1, catalytic subunit, alpha isoform
RAD23A	D21235	25.40	2.87	10.69	8.84	0.0002	2.37 RAN binding protein 2, like 1
PPF1CA	S57301	169.20	27.80	71.23	38.15	0.0001	2.37 scaffold attachment factor B
RANBP2L1	AF02086	23.00	3.94	9.69	7.59	0.0003	2.36 H2K binding factor-2
SAFB	U72355	10.40	2.30	4.39	3.73	0.0014	2.36 KluA0187 gene product
KU40082	D43949	87.60	14.88	26.54	18.19	0.0012	2.36 proteasome (prosome, macropain) 26S subunit, non-ATPase, 3
LOC51580	D14041	18.00	4.80	7.62	5.36	0.0009	2.36 v-src avian mesenchymal virus oncogene homolog 2
PDHB	D90086	21.80	3.70	9.23	8.40	0.0005	2.36 hec domain and RLD 2
KU40187	D80009	15.80	4.32	6.69	4.52	0.0047	2.36 Human clone 23533 mRNA sequence
PSMD3	D67025	15.80	1.30	6.69	4.48	0.0000	2.36 KluA1055 protein
DKFZP56424	AL080119	11.80	3.19	5.00	3.42	0.0044	2.36 hypothetical protein FL20259
MYC12	J03069	11.90	2.17	5.00	4.47	0.0008	2.35 KluA0252 protein
HERC2	AF041080	31.40	7.70	13.31	9.20	0.0024	2.35 interchromatin particle protein, 15 kDa
UNK_U78273	U78273	13.60	1.14	5.77	4.46	0.0000	2.35 DKFZP564A122 protein
KU41055	A0028078	17.40	3.65	7.38	5.80	0.0009	2.35 DKFZP564A122 protein
FL20259	W27545	15.40	3.78	5.69	3.68	0.0058	2.35 DKFZP564A122 protein
KU40252	D87440	13.20	4.78	6.89	5.16	0.0080	2.35 DKFZP564A122 protein
SCS1B	A003313	15.20	4.60	6.46	4.81	0.0020	2.35 DKFZP564A122 protein
DKFZP564A12	W26946	24.60	6.07	10.46	8.01	0.0020	2.35 DKFZP564A122 protein
PABPC4	U75066	17.00	3.67	7.23	4.59	0.0011	2.35 poly(A)-binding protein, cytoplasmic 4 (inducible form)
SIF2-28	U95511	34.00	5.70	14.46	8.72	0.0002	2.35 calcium and integrin binding protein (DNA-dependent protein kinase interacting protein)
DUT	U31900	30.00	2.45	12.77	7.53	0.0000	2.35 dUTP pyrophosphatase
TAL1	D84015	13.00	2.74	5.54	4.03	0.0009	2.35 TIA1 cytosolic granule-associated RNA-binding protein-like 1
DHPS	U26266	20.20	6.02	8.62	4.84	0.0014	2.34 deoxyhypusine synthase
KU40788	A0018331	22.00	6.20	9.38	7.35	0.0058	2.34 growth arrest and DNA-damage-inducible 34
UNK_AJ22187	AJ221875	11.00	2.35	4.69	3.04	0.0010	2.34 KluA0788 protein
TNP21	L40397	11.00	2.92	4.69	3.59	0.0039	2.34 Homo sapiens mRNA for putative glucosyltransferase, partial cds
MAPKBP3	A002899	10.80	2.17	4.62	2.84	0.0008	2.34 intramembrane trafficking protein
UNK_AL04995	AL049951	16.20	3.70	6.52	4.27	0.0016	2.34 mitogen-activated protein kinase B interacting protein 3
DDX16	A001149	28.60	8.99	12.23	8.53	0.0009	2.34 Homo sapiens mRNA; cDNA DKFZ564Q012 (from clone DKFZ564Q012)
ERCC5	L20048	10.60	2.07	4.54	2.99	0.0005	2.34 exonin repair cross-complementing rodent repair deficiency, complementation group 5 (keroderm pigmentum complementation group 5) Cockayne syndrome
RALY	L36896	10.60	1.67	4.54	2.76	0.0001	2.34 RNA-binding protein (nucleolar)
SLC20A1	L36899	13.80	3.49	5.82	4.41	0.0031	2.34 solute carrier family 20 (phosphate transporter), member 1
KU41080	A0028003	59.60	3.78	25.82	17.26	0.0003	2.33 KluA1080 protein; Cdk5-associated, gamma-scadin ear containing, ARF-binding protein 2
RNU6	U00001	13.80	3.65	5.86	4.10	0.0043	2.33 nucleolar rRNA
CHC1L	AF062219	16.80	3.42	7.22	4.34	0.0008	2.33 nitrogen fixation cluster-like
FOXO1A	AF022865	26.80	4.92	11.54	8.52	0.0004	2.32 chromosome condensation 1-like
FSTL1	D99307	53.90	14.84	23.08	21.23	0.0068	2.32 fibulin-like 1
UNK_AL04945	AL049450	13.40	3.85	5.77	3.59	0.0068	2.32 Homo sapiens mRNA; cDNA DKFZ568B192 (from clone DKFZ568B192)
ARGAP41	U02570	16.60	5.13	7.15	5.84	0.0094	2.32 Rho GTPase activating protein 1
IARS	U04953	11.60	2.70	5.00	3.49	0.0019	2.32 isoleucine-tRNA synthetase
PRKCD	D10495	11.60	2.07	5.00	3.56	0.0003	2.32 protein kinase C, delta

Table 7

RNF3	W23793	14.80	3.70	6.39	4.05	0.0030	2.33 ring finger protein 3
TRIM	AJ224878	55.40	15.68	23.52	21.38	0.0068	2.33 T-cell receptor interacting molecule
DNPA	U63547	22.80	5.89	9.85	8.47	0.0039	2.33 Homo sapiens PAK2 mRNA, complete cds
IGLQ	U53025	48.80	8.78	21.06	16.76	0.0004	2.32 hepatitis delta antigen-interacting protein A
NARS	X57869	13.00	2.92	5.62	4.23	0.0015	2.32 immunoglobulin lambda locus
RBM31P	D62351	12.40	27.81	52.92	47.83	0.0022	2.31 sparganglionic-RNA synthetase
NFATC3	L41067	14.40	1.34	6.23	4.38	0.0000	2.31 RNA binding motif, single stranded interacting protein 1, pseudogene
KNS2	L04733	31.40	6.50	13.62	10.18	0.0009	2.31 nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
RGS2	L13463	23.00	11.34	23.00	16.58	0.0011	2.30 kinesin 2 (80-70kD)
KIAA0682	AB011482	44.40	10.06	19.31	11.39	0.0017	2.30 regulator of G-protein signalling 2, 24kD
RING1	Z14000	21.40	3.36	9.31	6.13	0.0001	2.30 ring finger protein 1
PIGH	L19785	12.20	1.30	5.31	3.71	0.0000	2.30 phosphatidylinositol glycan, class H
COPIEB	AF001461	15.20	4.15	6.82	5.03	0.0051	2.30 core promoter element binding protein
KIAA0346	AB002344	42.40	9.07	18.48	12.86	0.0011	2.30 KIAA0346 protein
PRKRI	AB055508	16.60	3.85	7.23	4.19	0.0020	2.30 protein-kinase, interferon-inducible double stranded RNA dependent inhibitor
ALOX5AP	AB062222	97.00	23.16	42.31	28.04	0.0023	2.29 arachidonate 5-lipoxygenase-activating protein
FCGR2B	M28596	25.20	6.18	11.00	8.36	0.0028	2.29 Fc fragment of IgG, low affinity Iib, receptor for (CD32)
CD2	M16336	18.20	3.03	7.08	5.32	0.0007	2.28 CD2 antigen (p50), sheep red blood cell receptor
NK4	AF031872	19.00	5.10	8.31	6.82	0.0047	2.28 natural killer cell transcript 4
VAMP2	AL050223	14.60	2.51	6.38	4.35	0.0003	2.28 vesicle-associated membrane protein 2 (synaptobrevin 2)
A2LP	U07871	10.20	2.28	4.46	3.43	0.0017	2.28 ataxin 2 related protein
SHARCA2	D35155	45.40	11.63	20.31	13.36	0.0032	2.28 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
K1-157	U77327	21.60	3.21	9.46	5.98	0.0001	2.28 intracellular antigen detected by monoclonal antibody Ki-1; intracellular hyaluronan-binding protein
KIAA0435	AB007898	29.90	6.39	12.54	9.58	0.0017	2.28 putative L-type neutral amino acid transporter
NBD4	AF072250	21.40	5.81	9.38	6.70	0.0051	2.28 methyl-CpG binding domain protein 4
CHD3	U91543	36.20	3.96	5.92	4.18	0.0027	2.28 KRA8-associated protein 1
NBCD3	AF047472	26.40	5.13	11.62	8.57	0.0025	2.28 chromobox homolog 1 (Drosophila HP1 beta)
GLZ	AB359953	16.60	3.97	7.31	4.53	0.0005	2.28 hyaluronate synthase A dihydrogenase, type II
POLR2B	K63563	13.90	3.56	6.08	4.55	0.0002	2.27 chromodomain helicase DNA binding protein 3
DNM2	L25883	22.00	6.52	9.69	8.22	0.0068	2.27 BUB3 (nucleating inhibited by benzimidazole 3, yeast) homolog
P23	L24804	22.00	4.12	8.89	7.85	0.0007	2.27 nuclear receptor co-repressor 2
PPPIR2	U95735	11.00	1.22	4.85	2.97	0.0000	2.27 glucocorticoid-induced leucine zipper
SEC24B	AF08111	30.00	6.89	13.23	9.45	0.0019	2.27 polymerase (RNA) II (DNA directed) polypeptide B (140kD)
UNK_L25378	L29378	18.00	1.87	6.62	5.20	0.0001	2.27 protein phosphatase 1, regulatory (inhibitor) subunit 2
CEBPB	X52560	17.60	3.21	7.77	5.86	0.0005	2.27 SEC24 (S. cerevisiae) related gene family, member B
RBL2	X74594	13.40	2.70	5.92	4.11	0.0008	2.27 Homo sapiens (clone 3.5-1) MHC class I mRNA fragment
PRPSAP1	D61391	12.00	2.35	5.31	3.73	0.0007	2.27 CCAAT/enhancer binding protein (C/EBP), beta
KIAA0191	D83776	36.00	10.42	15.92	12.19	0.0074	2.26 retinoblastoma-like 2 (p130)
TIEG	S81439	23.80	7.19	10.34	9.02	0.0097	2.26 phosphoribosyl pyrophosphate synthetase-associated protein 1
H-PLK	M55422	13.20	3.49	5.85	5.54	0.0058	2.26 TGF-beta inducible early growth response
CDC16	U18291	39.40	11.33	17.46	13.25	0.0072	2.26 TGF-beta related zinc finger protein
CEBPD	M83867	53.80	5.69	23.77	17.62	0.0001	2.26 Kruippel-related zinc finger protein
STAT2	U18871	13.00	3.61	5.77	4.46	0.0061	2.26 CDC16 (cell division cycle 16, S. cerevisiae, homolog)
UNK_D28423	D28423	31.20	8.04	13.85	9.81	0.0040	2.26 T cell receptor alpha locus
KIAA0594	AB011166	11.60	2.70	5.15	3.26	0.0022	2.25 CCAAT/enhancer binding protein (C/EBP), delta
UNK_A055910F	AB59108	11.60	2.70	5.15	3.26	0.0022	2.25 signal transducer and activator of transcription 2, 113kD
IL17R	U56917	79.80	18.50	35.48	28.69	0.0022	2.25 Cluster/ind D28423; Human mRNA for pre-mRNA splicing factor SpR20, 5'UTR sequence from the 5' cap to the start codon.
UNK_U53588	U53588	34.60	10.29	15.38	11.03	0.0087	2.25 Homo sapiens chromosome 19, cosmid R28379
NXF1	AJ132712	19.20	3.35	8.54	6.39	0.0004	2.25 Interleukin 17 receptor
UNK_W73822	W73822	10.20	1.79	4.54	3.31	0.0004	2.25 Cluster/ind U53588; Homo sapiens MHC class I region.
CLIC3	L28218	14.00	2.74	6.23	4.84	0.0007	2.25 nuclear RNA export factor 1
UNK_A0743554	A074354	45.80	7.56	20.38	14.13	0.0002	2.25 Cluster/ind W73822; zebrafish 10.1 Soares, J. et al. NHRH19W Homo sapiens cDNA clone IMAGE:344299 3', mRNA sequence.
STK17A	AB61743	21.60	5.46	9.82	7.82	0.0004	2.25 CDC-like kinase 2
KIAA0700	AB014600	26.80	7.44	11.85	7.82	0.0004	2.25 Homo sapiens cDNA FLJ11365 flc, clone HEMBA1002692
POU2AF1	Z81914	50.90	6.11	22.54	15.50	0.0000	2.25 serine/threonine kinase 17a (apoptosis-inducing)
CDK25B	Z81914	29.00	3.32	12.92	9.00	0.0000	2.25 POU domain, class 2, associating factor 1
SKIVL2	U06817	29.00	5.83	12.92	8.45	0.0008	2.24 cell division cycle 25B
							2.24 superkiller viral-like activity 2 (S. cerevisiae homolog) like

Table 7

KAA0169	D75991	12.60	2.07	5.52	4.13	0.0003	2.24 KAA0169 protein
SOD1	X02317	21.40	3.03	9.54	7.10	0.0024	2.24 superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))
STAM	U43899	27.60	5.50	12.31	11.69	0.0021	2.24 signal transducing adaptor molecule (SH3 domain and ITAM motif) 1
HRP4	U03532	33.80	8.76	15.08	10.24	0.0042	2.24 heterogeneous nuclear ribonucleoprotein M
CDC2L1	XZ1712	25.00	7.48	11.15	6.35	0.0095	2.24 cell division cycle 2-like 1 (PITSLRE proteins)
GALT	N60091	10.00	2.92	4.46	2.47	0.0035	2.24 galactose-1-phosphate uridylyltransferase
KATHB1	AF032432	21.20	4.27	9.46	6.09	0.0008	2.24 katanin p60 (WAO-containing) subunit B 1
IGHM	X58529	16.20	2.69	7.23	4.85	0.0002	2.24 immunoglobulin heavy constant mu
TRN-SR	AA192356	21.00	5.15	9.38	6.40	0.0031	2.24 transerlin-SR
NHP2L1	Z03840	58.00	10.20	25.92	19.73	0.0005	2.24 non-histone chromosome protein 2 (S. cerevisiae)-like 1
FYB	AF011852	11.00	1.87	4.92	2.66	0.0002	2.23 FYB-binding protein (FYB-120/130)
KAA0174	D75996	11.00	3.32	4.92	3.01	0.0098	2.23 KAA0174 gene product
UNK_AL031775	AL031775	11.00	1.87	4.92	2.29	0.0003	2.23 Cluster Int AL031775: Human DNA sequences from clone 30A3 on chromosome 6p22.1-22.3. Contains three novel genes, one similar to C. elegans Y63D3A.4
ZAP70	U05148	11.00	2.83	4.92	3.48	0.0041	2.23 zeta-chain (TCR) associated protein kinase (70 kD)
CHES1	U68723	13.40	2.51	6.00	4.60	0.0007	2.23 checkpoint suppressor 1
UNK_AA78004	AA78004	13.40	2.51	6.00	3.76	0.0005	2.23 Homo sapiens cDNA: FLJ21439 flt, clone COL04352
KAA1097	AB028020	52.20	4.76	23.39	13.91	0.0000	2.23 KAA1097 protein
ATP5A1	D11710	19.40	4.83	8.69	6.09	0.0034	2.23 ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, cardiac muscle
BAX	U19599	21.80	3.56	9.77	7.13	0.0003	2.23 BCL2-associated X protein
ARR32	AF106941	10.80	1.48	4.85	2.34	0.0000	2.23 arrestin, beta 2
ATM	U64555	18.00	5.79	8.08	5.65	0.0005	2.23 ataxia telangiectasia mutated (includes complementation groups A, C and D)
CA150	AF017769	14.40	2.70	8.46	4.88	0.0007	2.23 transcription factor CA150
MOF	AA170735	19.20	1.79	8.62	4.17	0.0000	2.23 member of MYST family histone acetyltransferases, homolog of Drosophila MOF
NUP	X0202045	15.60	4.72	7.00	4.34	0.0100	2.23 nuclear pore complex interacting protein
UNK_AF026811	AF026816	14.40	3.51	8.46	4.70	0.0031	2.23 Homo sapiens putative oncogene protein mRNA, partial cds
MTMR4	AB014547	24.00	3.32	10.77	7.78	0.0001	2.23 myotubularin related protein 4
SP3A3	X81769	13.20	3.11	5.92	4.70	0.0028	2.23 splicing factor 3a, subunit 3, 60kD
PIGC	D85418	43.00	10.68	19.31	10.51	0.0036	2.23 phosphatidylinositol glycan, class C
KAA0349	KAA0347	41.80	5.86	18.69	12.15	0.0001	2.23 KAA0349 protein
ZNF198	AJ224901	16.60	3.51	7.46	5.17	0.0013	2.22 zinc finger protein 198
TCF8	D125060	15.40	3.34	6.92	4.07	0.0018	2.22 transcription factor 8 (represses interleukin 2 expression)
AP1G2	A741833	23.00	3.39	5.85	4.24	0.0046	2.22 adaptor-related protein complex 1, gamma 2 subunit
PTPRE	X54134	23.60	6.58	10.62	7.77	0.0066	2.22 protein tyrosine phosphatase, receptor type, E
VHL	L15409	56.60	3.85	25.46	16.16	0.0000	2.22 von Hippel-Lindau syndrome
AF0301	AF002163	10.60	2.51	4.77	3.72	0.0028	2.22 adaptor-related protein complex 3, delta 1 subunit
HCDI	AA94623	25.80	3.83	11.82	9.20	0.0003	2.22 HCDI protein
MGP	W28510	14.00	2.45	6.31	4.71	0.0005	2.22 matrix Gla protein
IK	AJ005579	60.60	11.15	27.31	21.19	0.0007	2.22 IK cytokine, down-regulator of HLA II
KAA1028	AB028951	19.80	4.27	6.92	5.68	0.0014	2.22 KAA1028 protein
LYL1	M22637	32.60	6.11	14.69	7.09	0.0006	2.22 lymphoblastic leukemia derived sequence 1
STX10	AB015718	12.80	2.49	5.77	4.13	0.0008	2.22 serine/threonine kinase 10
DNK7P568H07	AL050060	50.00	10.84	22.54	17.91	0.0018	2.22 DNK7P568H07 protein
KAA0515	AB011087	18.60	5.41	8.38	5.52	0.0003	2.22 KAA0515 protein
USP1	AB014458	16.20	4.38	7.31	5.25	0.0056	2.22 ubiquitin specific protease 1
ARH1	AJ009771	13.00	2.83	6.77	4.78	0.0007	2.22 aridine (Drosophila) homolog, ubiquitin-conjugating enzyme E2-binding protein, 1
UNK_W26634	W26634	30.00	5.15	13.54	7.21	0.0003	2.22 Cluster Int W26634: 34610 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence:
AFH-GDIA	X69350	48.20	4.09	21.77	17.28	0.0001	2.21 Rho GTP dissociation inhibitor (GDI) alpha
HDA-C1	U50079	12.60	3.51	5.69	4.19	0.0067	2.21 histone deacetylase 1
XIP	AF029890	12.50	2.30	5.69	3.88	0.0005	2.21 histone deacetylase 1
KAA0365	AB002363	22.80	3.77	10.31	6.51	0.0002	2.21 KAA0365 gene product
PHF2	AB014562	49.00	13.40	22.15	16.12	0.0061	2.21 PHD finger protein 2
KAA0544	AB011116	14.80	3.77	6.69	4.44	0.0040	2.21 KAA0544 protein
ADA2B1	U76421	31.80	5.22	14.38	8.53	0.0002	2.21 adenosine deaminase, RNA-specific, B1 (homolog of rat RED1)
MCP	X59408	31.80	6.69	14.38	10.60	0.0014	2.21 membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)
KAA0117	AA887480	10.20	2.39	4.82	3.12	0.0025	2.21 KAA0117 protein
SFRS5	AL031681	17.00	1.87	7.69	5.78	0.0001	2.21 splicing factor, arginine/serine-rich 6
KAA0028	AB023143	26.00	4.64	11.77	8.79	0.0006	2.21 KAA0028 protein
ITPR3	U01062	15.80	3.03	7.15	4.08	0.0006	2.21 inositol 1,4,5-trisphosphate receptor, type 3
EIF5	AL080102	12.40	3.65	5.62	3.20	0.0092	2.21 eukaryotic translation initiation factor 5
NCL	M60958	49.60	6.98	22.46	14.28	0.0001	2.21 nucleolin
RASA1	M23379	14.60	3.71	6.62	4.05	0.0017	2.21 RAS GTP protein activator (GTPase activating protein) 1
PRC1	U78310	16.80	3.20	7.62	4.91	0.0017	2.21 pectadial (zebrin-like) homolog 1, containing BRCT domain
CDK2L1	D38451	24.60	4.39	11.15	6.57	0.0004	2.20 CDK2L1 (S. pombe) homolog
KAA1041	AB028864	15.60	2.51	7.08	3.26	0.0002	2.20 KAA1041 protein
ETR101	N69331	33.40	7.03	15.15	12.03	0.0029	2.20 immediate early protein
E2F4	X632140	17.80	3.03	8.09	5.44	0.0003	2.20 E2F transcription factor 4, p107/p130-binding
DGP	U62213	22.20	4.55	10.08	11.03	0.0046	2.20 D site of albumin promoter (albumin D-site) binding protein
TBA4	AB011169	14.40	3.56	6.54	3.89	0.0037	2.20 similar to S. cerevisiae SSIM
KAA1067	AB028990	75.20	12.50	34.15	23.68	0.0003	2.20 KAA1067 protein

Table 7

SP382	U41371	63.00	9.67	28.62	25.14	0.0007	2.20	splicing factor 3b, subunit 2, 145KD
DGSI	L77566	11.00	1.22	3.00	2.16	0.0000	2.20	DGeorge syndrome critical region gene DGS1
DUSP1	X68277.8	17.80	1.67	8.00	4.84	0.0000	2.20	dual specificity phosphatase 1
KAA00911	U73324	11.00	2.12	5.00	3.79	0.0009	2.20	KAA00911 protein
RABF	U73324	27.40	7.96	12.46	9.00	0.0085	2.20	RAB interacting factor
AB029034	U73324	20.80	3.56	9.46	6.79	0.0004	2.20	KAA1111 protein
MARF2	X42322	14.20	3.03	8.48	4.99	0.0017	2.20	microtubule-associated protein, RP/EB family, member 2
GRSF1	U07231	24.00	4.64	10.92	7.94	0.0009	2.20	G-rich RNA sequence binding factor 1
KAA00752	AB018295	12.00	3.39	5.46	3.99	0.0074	2.20	KAA00752 protein
PPP2R4	X73478	13.00	2.55	5.92	3.88	0.0008	2.19	protein phosphatase 2A, regulatory subunit B (ppr 53)
CFDP1	AB009285	31.40	5.13	14.31	9.01	0.0002	2.19	cathepsin D-like protein 1
DED	U51698	31.40	8.14	14.31	9.74	0.0047	2.19	apoptosis antagonizing transcription factor
PSMB7	D38048	23.80	6.50	10.85	6.18	0.0064	2.19	proteasome (prosome, macropain) subunit, beta type, 7
UNK_W32483	W32483	16.20	3.96	7.38	5.64	0.0036	2.19	Human DNA sequence from clone 243E3 on chromosome 1p36.21-36.33. Contains the alternatively spliced gene for Matrix Metalloproteinase in the Female Rb
EPPT0213-2	U94636	30.20	7.29	13.77	9.99	0.0032	2.19	cathepsin B (prosome, macropain) subunit, beta type, 7
CTPB	Y08683	19.40	4.72	8.85	5.49	0.0027	2.19	protein with polyglutamine repeat; calcium (Ca2+) homeostasis endoplasmic reticulum protein
UNK_AL02210	AL02210	19.40	5.50	8.85	6.47	0.0015	2.19	Cluster Ind AL022101: Homo sapiens DNA sequence from PAC 945024 on chromosome 1p36.1-36.2. Contains a gene for a Heterogenous Nuclear Ribonucle
MDM3	U68019	17.20	3.11	7.85	6.43	0.0009	2.19	MDM2 (p107) interacting protein 3
CTB	D13827	42.80	3.56	19.54	12.86	0.0007	2.19	chaperonin containing TCP1, subunit 8 (beta)
DMF2P56Q24	AL050015	12.80	3.63	5.85	3.53	0.0078	2.19	translocase of inner mitochondrial membrane 17 (yeast) homolog A
GNG10	U31383	12.80	3.03	5.85	4.45	0.0030	2.19	guanine nucleotide binding protein 10
KAA0073	D38552	22.40	3.13	10.23	7.73	0.0002	2.19	KAA0073 protein
SFRS10	U68063	12.80	2.39	5.85	3.72	0.0006	2.19	splicing factor, arginine/serine-rich (transducer 2 Drosophila homolog) 10
GAT3A	X58072	38.20	6.53	17.46	13.54	0.0007	2.19	GAT3A-binding protein 3
RNG	AF001549	28.60	7.02	13.08	9.95	0.0037	2.19	RNA polymerase I transcription factor RRN3
PRPE	AF042365	71.90	12.36	32.85	26.29	0.0007	2.19	perlecan (perlecan, perlecanin) E (cytoplasmic E)
SATB1	M97287	19.00	4.00	8.69	3.99	0.0016	2.19	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associated DNAs)
DKFZF586G01	AL060126	185.60	39.68	84.92	45.01	0.0015	2.19	DKFZF586G01 protein
IMPDI1	J05272	62.20	15.25	28.46	26.51	0.0051	2.19	IMP (inositol monophosphate) dehydrogenase 1
PIG7	AF010312	55.80	13.05	25.54	17.76	0.0027	2.19	LPS-induced TNF-alpha factor
ARRH	Z35227	12.60	2.30	5.77	3.52	0.0005	2.18	ras homolog gene family, member H
DEK	X64229	18.80	3.11	7.69	6.22	0.0010	2.18	DEK oncogene (DNA binding)
HSU3209	U53209	17.80	2.59	8.15	4.69	0.0001	2.18	transducer-2 alpha (Tira-2 alpha)
KAA0554	AB011126	52.20	11.82	23.92	16.21	0.0022	2.18	KAA0554 protein
BIRC3	U45878	24.00	4.00	11.00	7.87	0.0004	2.18	baculoviral IAP repeat-containing 3
TRF8Q	X00437	65.60	18.34	30.08	23.73	0.0076	2.18	T cell receptor beta locus
LCAL8	L78132	10.40	2.19	4.77	3.24	0.0014	2.18	lectin, galactoside-binding, soluble, 8 (galactin 8)
SPN	AF049460	28.00	7.52	11.92	7.86	0.0086	2.18	suppressor (nuclear deformed autoregulatory factor-1 (DEAF-1)-related)
BAF3A	AF041474	43.20	8.61	19.85	12.34	0.0009	2.18	BAF53
NAP1L1	M85687	14.40	2.81	6.82	4.09	0.0005	2.18	nucleosome assembly protein 1-like 1
SSH3BP1	AF006518	44.20	6.76	20.31	16.78	0.0006	2.18	spectin SH3 domain binding protein 1
FUS1	AF006579	122.20	23.75	56.23	33.99	0.0008	2.17	membrane component, chromosome 11, surface marker 1
M1151	Z48042	11.20	1.64	5.15	3.61	0.0001	2.17	lipidome-related function protein 4-1
TRF4	AB00754	11.20	2.28	5.15	3.36	0.0011	2.17	ectopic viral integration site 2B
EV2B	M00030	24.40	6.23	11.23	7.06	0.0045	2.17	perlecan (perlecan, perlecanin) E (cytoplasmic E)
PIE	AF042386	13.20	3.03	6.08	4.54	0.0027	2.17	Cluster Ind AB08712: w57G5.x1 Soares, NFL, T, GBC, S1 Homo sapiens cDNA clone IMAGE:235988 3', mRNA sequence.
UNK_AB08712	AB08712	14.20	2.28	6.54	4.27	0.0002	2.17	adaptor-related protein complex 2, beta 1 subunit
AP2B1	M04175	33.40	7.09	15.38	10.67	0.0016	2.17	protein tyrosine phosphatase, receptor type, C-associated protein
PTPRCAP	X97257	33.40	4.16	15.38	8.49	0.0000	2.17	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9
PSMD9	A047155	35.40	8.08	16.31	13.73	0.0032	2.17	splicing factor, arginine/serine-rich 2
SFRS2	X75755	20.20	3.96	9.31	5.79	0.0009	2.17	choline kinase-like
CHKL	AL096780	21.20	5.38	9.77	7.32	0.0045	2.17	KAA0128 protein; sepiin 2
KAA0128	AB19842	10.00	1.41	4.52	2.47	0.0001	2.17	splicing factor 3a, subunit 2, 56KD
SF3A2	L21990	20.00	3.67	9.23	6.73	0.0008	2.17	absent in melanoma 1
AMH	U83115	16.00	4.06	7.38	4.65	0.0044	2.17	C-terminal binding protein 1
CTBP1	U07408	13.00	3.54	6.00	4.93	0.0071	2.17	synaptophysin-like protein
SYPL	X88194	12.00	1.87	5.54	3.43	0.0002	2.17	transmembrane protein 4
TUE44	AB015331	12.00	2.12	5.54	3.71	0.0005	2.17	Homo sapiens clone 24432 mRNA sequence
UNK_AF07053	AF07053	14.00	2.92	6.46	4.07	0.0013	2.18	KAA0018 gene product
KAA0018	N2685	29.80	4.44	13.77	8.28	0.0001	2.18	small nuclear ribonucleoprotein polypeptide F
SRPF	X93205	24.80	3.56	12.85	7.02	0.0001	2.18	aridyl (N-arginine diacyl conase)
UNK_AL04112	AL04112	21.80	5.89	10.08	5.79	0.0064	2.18	Cluster Ind AL041121: DKFZP434D0316.31 434 (synonym: hts3) Homo sapiens cDNA clone DKFZP434D0316 3', mRNA sequence.
NP220	D83032	14.80	3.27	8.85	4.34	0.0003	2.18	nuclear protein
ZNF716	AF062346	14.80	3.27	8.85	4.47	0.0020	2.18	tumor necrosis factor receptor superfamily, member 10b
THFRSF108	AF016266	26.50	5.77	12.31	8.68	0.0019	2.16	Rho GDP dissociation inhibitor (GDI) beta
APH-GDB8	X69549	35.40	6.27	16.38	11.14	0.0005	2.16	B-cell CLL/lymphoma 6 (dnc finger protein 51)
BCL6	U00115	11.80	3.03	5.46	3.69	0.0048		

Table 7

H1FX	D64142	23.60	4.34	10.92	6.55	0.0005	2.16 H1 histone family, member X
SP3B1	AF054284	11.80	2.39	5.48	4.14	0.0014	2.16 splicing factor 3b, subunit 1, 155kD
ETFA	J04058	34.20	9.80	15.85	13.19	0.0085	2.16 electron-transfer-flavoprotein, alpha polypeptide (glutamic adducin 8)
TIC	U63127	24.40	4.72	11.31	7.45	0.0009	2.16 SEC7 homolog
ADAR	X79448	81.00	17.71	37.54	28.80	0.0020	2.16 adenosine deaminase, RNA-specific
TRIP3	L40410	13.60	3.85	6.31	3.81	0.0064	2.16 thyroid hormone receptor interactor 3
LCP2	U20158	11.60	2.19	5.38	3.84	0.0009	2.15 lymphocyte cytosolic protein 2 (SH2 domain-containing leukocyte protein of 78kD)
KIA0242	Y06764	87.80	25.42	40.77	29.33	0.0092	2.15 KIA0242 protein
HSP48	Y06371	10.60	2.41	4.92	3.23	0.0024	2.15 heat shock 70kD protein 8
FNAL	AJ008112	25.00	4.30	11.82	9.40	0.0009	2.15 lamin-like
LY75	AF011333	86.40	16.68	40.15	27.88	0.0009	2.15 lymphocyte antigen 75
ITF3	X57352	19.20	5.02	8.92	8.30	0.0075	2.15 interferon induced transmembrane protein 3 (18U)
ILF3	U10324	42.20	6.76	19.52	14.49	0.0004	2.15 interleukin enhancer binding factor 3, 50kD
MAP3K4	AF002715	18.20	4.49	8.46	5.61	0.0039	2.15 mitogen-activated protein kinase kinase kinase 4
KIA00442	AB007802	16.20	2.68	7.54	4.52	0.0003	2.15 KIA00442 protein
COX11	U27270	14.20	3.63	6.62	4.13	0.0048	2.15 COX11 (yeast) homolog, cytochrome c oxidase assembly protein
RUNX2	Z35278	12.20	2.95	5.69	4.79	0.0045	2.14 over-expressed transcription factor 3
UNK_AL050144	AL050146	28.20	5.72	12.23	8.32	0.0020	2.14 Homo sapiens clone 25120 mRNA sequence
UNK_W25988	W25988	16.80	2.59	7.85	5.34	0.0003	2.14 Homo sapiens cDNA FL20332 fls. clone XA1110877
SORL1	Y08110	36.40	2.66	17.00	9.33	0.0000	2.14 sortilin-related receptor, L (OLR class) A repeats-containing
KIA0138	D59028	66.80	19.36	31.23	23.58	0.0098	2.14 KIA0138 gene product
DM1	AF025612	10.20	2.39	4.77	2.92	0.0029	2.14 similar to S. pombe dmi+
LOC51317	X83528	10.20	2.28	4.77	3.78	0.0028	2.14 hypothetical protein
KIA00766	AB018309	32.40	4.28	15.15	7.70	0.0000	2.14 TATA box binding protein (TBP)-associated factor, RNA polymerase II, L, 28kD
SPTLC1	Y06885	19.40	3.58	9.08	5.56	0.0022	2.14 KIA00766 gene product
VP526	AF054179	12.00	2.92	5.62	4.35	0.0042	2.14 serine palmitoyltransferase, long chain base subunit 1
LMO4	U24578	33.20	9.28	15.54	10.52	0.0079	2.14 vacuolar protein sorting 28 (yeast homolog)
TSC2	X75621	18.40	5.27	8.62	7.29	0.0101	2.14 LIM domain only 4
GP32	U28963	29.40	7.80	13.77	10.07	0.0062	2.14 tuberous sclerosis 2
LOC51172	AF052111	40.40	11.44	18.92	11.54	0.0065	2.14 G protein pathway suppressor 2
DNF2P56A15	AB57673	26.60	7.50	12.46	10.32	0.0092	2.13 N-acetylglucosaminyl-1-phosphodiester alpha-N-acetylglucosaminidase
SFRS5	X00026	168.60	5.81	12.48	8.47	0.0020	2.13 DKFZP566J153 protein
PIP2	U070533	169.60	26.59	51.38	36.86	0.0039	2.13 splicing factor, arginine/serine-rich 5
EEF10	Z21507	76.40	4.39	12.38	7.79	0.0003	2.13 tumor necrosis factor alpha-inducible cellular protein containing leucine zipper domains; Huntingtin interacting protein L; transcription factor IIA-interacting protein
UNK_W21675	W21675	10.00	2.55	4.69	2.46	0.0031	2.13 eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)
LOC58977	AB51368	31.80	5.31	14.92	7.24	0.0003	2.13 Cluster Int W21675: 38k3 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence.
UNK_AL049306	AL049309	35.40	6.91	16.62	12.69	0.0012	2.13 hypothetical protein 24633
PP4AC	X02218	37.20	9.63	17.46	12.31	0.0054	2.13 protein phosphatase 4 (formerly X), catalytic subunit
SAS10	A126004	13.60	2.79	5.38	4.57	0.0016	2.13 cleaver of blending 10
CD8	X60992	15.40	3.91	7.23	5.64	0.0054	2.13 CD8 antigen
RBM31	X71484	55.20	9.68	23.92	20.75	0.0011	2.13 KIA0100 gene product
UNK_W72239	W72239	224.20	54.21	105.38	65.37	0.0038	2.13 RNA binding motif, single stranded interacting protein 1
KIA1116	AB028039	10.80	2.26	5.08	3.28	0.0016	2.13 Homo sapiens mRNA; cDNA DKFZP434M152 (from clone DKFZP434M152)
OXA1L	X80695	18.80	5.36	9.31	6.02	0.0068	2.13 KIA1116 protein
ASMTL	AA889799	19.60	5.41	9.23	7.07	0.0091	2.13 outsize (cytochrome c) assembly 1-like
MD3019	AL078541	22.20	4.60	8.68	7.42	0.0017	2.12 polyserotonin O-methyltransferase-like
CREM3	AF046059	12.40	2.70	5.85	4.74	0.0028	2.12 photolabile protein MDS019
DAP3	U18321	12.40	3.29	5.85	5.37	0.0095	2.12 cytochrome receptor-like molecule 9
NME3	U29656	11.40	3.21	5.39	3.43	0.0095	2.12 death associated protein 3
ITGAM	D49728	32.40	4.04	15.31	10.19	0.0001	2.12 non-metastatic cells 3, protein expressed in
CD32	U07434	14.00	2.45	6.62	4.39	0.0006	2.12 integrin, alpha M (complement component receptor 3, alpha; also known as CD11b (p170), macrophage antigen alpha polypeptide)
ORF2	U07802	16.60	3.65	7.85	5.68	0.0024	2.12 cytoskeleton-associated protein 1
USA-CYP	AF018371	117.00	31.44	55.31	37.35	0.0068	2.12 2-5' oligoadenylate synthetase 2
KIA00831	AB020638	46.20	8.35	21.85	11.88	0.0005	2.12 bivariate response factor 2 (EGF-response factor 2)
JAK1	K04174	14.80	2.49	7.00	4.12	0.0003	2.11 KIA00831 protein
LOC51614	AF091065	14.80	1.92	7.00	5.39	0.0003	2.11 Janus kinase 1 (a protein tyrosine kinase)
UNK_AB09060C	AB090603	30.40	7.16	14.38	12.80	0.0051	2.11 hypothetical 43.2 kD protein
SMARCE1	AF035262	53.80	7.01	25.46	19.76	0.0004	2.11 Cluster Int AB090603: wnt110.01 NC1_CGAP_U2 Homo sapiens cDNA clone IMAGE2443339 3' mRNA sequence.
NL64	D38255	73.60	19.48	34.85	21.49	0.0062	2.11 SYR333P related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1
UNK_AL04581	AL045811	92.60	6.59	43.95	40.19	0.0009	2.11 serine/threonine kinase regulatory protein related
RNAHP	U043255	34.60	9.56	16.38	11.00	0.0079	2.11 KIA00579 protein
SNRPN	U11303	11.20	3.19	4.82	3.12	0.0093	2.11 Homo sapiens mRNA; cDNA DKFZP434C0814 (from clone DKFZP434C0814)
KIA0118	D29958	33.40	5.18	15.85	8.84	0.0002	2.11 RNA helicase-related protein
KIA00983	AB014563	15.40	2.70	7.31	4.82	0.0008	2.11 small nuclear ribonucleoprotein polypeptide N
TP53	M22898	15.40	4.04	7.31	5.31	0.0063	2.11 KIA00983 gene product
							2.11 tumor protein p53 (Li-Fraumeni syndrome)

Table 7

BCS1L	AF038195	24.80	3.70	11.77	5.18	0.0001	2.11 BCS1 (yeast homologue-like)
PSMD1	D44466	50.40	11.33	23.92	15.96	0.0026	2.11 proteasome (prosome, macropain) 26S subunit, non-ATPase, 1
OXCT	U62961	16.20	4.15	7.89	5.47	0.0055	2.11 3-oxoacid CoA transferase
DNFZP47E21	AL050261	30.50	6.80	14.54	10.28	0.0028	2.10 DNFZP47E21110 protein
LYPLA1	AF081281	20.40	5.18	9.69	6.07	0.0051	2.10 lysophospholipase 1
UNK_L40402	L40402	17.00	3.32	8.08	6.86	0.0022	2.10 Homo sapiens mRNA, cDNA DNFZP434D0935 (from clone DNFZP434D0935)
KAAL1049	AB028972	17.80	2.28	8.46	5.83	0.0002	2.10 Homo sapiens mRNA, cDNA DNFZP434D0935 (from clone DNFZP434D0935)
ABLIM	U01183	205.80	34.78	97.85	63.53	0.0005	2.10 actin binding LIM protein 1
H5F2	M65217	11.80	2.59	5.62	3.35	0.0022	2.10 heat shock transcription factor 2
RELA	L19087	11.80	2.39	5.62	3.91	0.0015	2.10 v-rel avian retrovirus oncogene homolog A (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (p65))
NFKB1	M58603	25.20	4.09	12.00	8.02	0.0004	2.10 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
UNK_U50535	U50535	38.60	6.54	18.38	10.87	0.0004	2.10 Human BRCA2 region, mRNA sequence CG008
SULT1A1	X78293	82.20	11.30	39.15	26.38	0.0003	2.10 sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1
DNFZP56A08	W21827	26.00	6.44	12.38	10.17	0.0057	2.10 DNFZP56A082 protein
DNFZP47E10	AL050260	21.80	5.78	10.38	9.40	0.0088	2.10 DNFZP47E1010 protein
DNFZP2206	Z82206	17.40	1.95	8.39	5.72	0.0001	2.10 Cluster hot Z82206: Human DNA sequence from clone RP3-370422 on chromosome 22. Contains part of the gene for ubiquitin-cytochrome c reductase non-s
CLN3	U32680	26.80	3.63	12.77	8.63	0.0013	2.10 cereoid-liquidus factor, neuronal 3, juvenile (Batten, Scheinberg-Vogt disease)
KAAL0818	AL080108	13.40	2.70	5.38	4.05	0.0013	2.10 CD3D antigen, delta polypeptide (TIT3 complex)
PCOLCE	U58046	10.00	2.35	4.77	4.40	0.0081	2.10 procollagen (type III) N-endopeptidase
UNK_AJ133111	AJ133115	38.90	9.74	17.40	13.20	0.0072	2.10 Homo sapiens mRNA for TSC-22-like protein
WEE1	W28575	40.50	10.04	19.38	13.52	0.0047	2.09 DNFZP727M231 protein
FLJ20500	AA522530	17.40	3.91	8.31	5.60	0.0027	2.09 weel1+ (S. pombe) homolog
CD14	X08882	29.80	6.65	14.23	9.02	0.0025	2.09 hypothetical protein
GOLGA1	U51587	18.20	1.02	8.69	5.48	0.0001	2.09 CD14 antigen
UNK_W28851	W28851	30.50	5.94	14.62	9.61	0.0011	2.09 golgi antigen, polig subfamily a, 1
VPSA58	AJ133421	13.20	3.27	6.31	3.90	0.0046	2.09 endocase of inner mitochondrial membrane 44 (yeast) homolog
PRKCB1	X07109	40.40	5.55	19.31	12.29	0.0002	2.09 ESTs, weakly similar to ALU7_HUMAN ALU SUBFAMILY SQ SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]
DDX3	U05553	167.20	28.74	79.92	54.70	0.0006	2.09 vesicular protein sorting 458 (yeast homolog)
HSA9781	W07859	14.80	3.42	7.08	4.90	0.0032	2.09 protein kinase C, beta 1
KIA0275	D87465	15.80	2.88	7.46	5.14	0.0009	2.09 DEAD/H (Asp-Glu-Ala-Asp/Gln) box polypeptide 3
KIA0438	AB007888	15.60	3.21	7.48	5.08	0.0017	2.09 putative dimethyladenosine transferase
PRNP	U29185	23.80	2.17	11.38	6.13	0.0000	2.09 KIA0275 gene product
HDC6	A011972	47.40	8.30	22.69	17.73	0.0012	2.09 KIA0438 gene product
GRN	AF035008	21.20	3.69	10.15	6.56	0.0009	2.09 piron protein (p27-30) (Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia)
KIA0244	D87685	44.80	7.19	21.46	14.91	0.0005	2.09 histone deacetylase 6
UBE2N	D88004	11.40	1.34	5.46	3.89	0.0002	2.09 IL2-inducible T-cell kinase
HAX1	U68586	17.00	3.00	8.15	5.49	0.0007	2.09 ubiquitin-conjugating enzyme E2N (homologous to yeast UBC13)
FCGR2A	M31932	37.20	6.72	17.85	13.27	0.0011	2.09 HST1 binding protein
SP2	D28588	18.60	2.07	8.92	6.47	0.0002	2.09 Fc fragment of IgG, low affinity IIa, receptor for (CD32)
CREB1	M27891	103.00	17.76	49.46	29.88	0.0005	2.08 S22 transcription factor
ABR	U01147	30.40	7.89	14.62	10.53	0.0004	2.08 cAMP responsive element binding protein 1
ANPEP	M22324	23.20	4.27	11.15	8.13	0.0011	2.08 active BCR-related gene
GABBR1	AJ275028	10.40	2.30	5.00	3.08	0.0025	2.08 alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)
KIA0179	D60001	20.00	4.74	9.62	6.96	0.0041	2.08 gamma-aminobutyric acid (GABA) B receptor, 1
KIA0630	AB014530	40.80	7.16	19.62	14.74	0.0010	2.08 KIA0630 protein
SNAP	AY020556	20.80	2.66	10.00	5.42	0.0001	2.08 thyroid hormone receptor coactivating protein
UNK_AL03128	AL031282	54.40	8.53	26.15	17.09	0.0004	2.08 Cluster hot AL031282: Human DNA sequence from clone 263E3 on chromosome 1 p36.21-36.33. Contains the alternatively spliced gene for Matrix Metallo
EED	AF080227	68.20	11.43	32.85	20.82	0.0027	2.08 embryonic ectoderm development
ARRGEE1	Y09160	40.40	3.97	19.46	13.89	0.0001	2.08 Rho guanine nucleotide exchange factor (GEF) 1
PFP5K2B	U65245	18.20	4.76	8.77	6.86	0.0073	2.08 phosphatidylinositol-4-kinase, type II, beta
UNK_H36842	H36842	18.20	4.96	8.77	6.31	0.0061	2.08 Homo sapiens cDNA FLJ10207/rt, clone HEMBA1005475
SDHA	L21938	17.40	3.21	8.30	5.58	0.0009	2.08 succinate dehydrogenase complex, subunit A, flavoprotein (F6)
BTG2	U77049	13.40	3.03	6.46	4.16	0.0030	2.07 BTG family, member 2
RPC	U11651	13.40	3.65	6.46	3.97	0.0079	2.07 RNA 3'-terminal phosphate cyclase
KIA0135	D50925	64.80	8.65	31.15	22.74	0.0004	2.07 KIA0135 protein
UNK_AJ00978	AF099787	12.60	1.52	6.08	3.30	0.0000	2.07 KIA0237 protein
HLA-G	M30683	36.20	8.47	17.46	11.84	0.0038	2.07 Homo sapiens T cell receptor beta chain (TCRB) mRNA, partial cds
STX7	U77942	11.80	2.49	5.69	3.54	0.0019	2.07 HLA-G histocompatibility antigen, class I, G
MAEA	AF084928	11.00	2.35	5.31	4.35	0.0032	2.07 macrophage erythroblast effector
SFRS311	M74002	11.00	2.45	5.31	4.35	0.0032	2.07 splicing factor, arginine/serine-rich 11
ARL7	M0216911	10.20	0.84	4.92	3.62	0.0002	2.07 ADP-ribosylation factor-like 7
IGHD	M02882	10.20	2.39	4.92	3.62	0.0040	2.07 immunoglobulin heavy constant delta
TPM4	X05276	10.20	2.39	4.92	3.28	0.0035	2.07 tropomyosin 4

Table 7

UNK_A018098	258.20	51.21	123.69	70.16	0.0013	2.07 Cluster lnc A018098; ccd8b1.1.1 Soares; NHT Homo sapiens cDNA clone IMAGE:642173.3; mRNA sequence.
GZ2-1	17.20	4.71	8.31	6.21	0.0088	2.07 TNF-induced protein
PAC2	69.60	11.95	33.21	17.07	0.0005	2.07 rat-recler C3 sodium iono subunit 2 (mo family, small GTP binding protein Rac2)
SLC2A1	28.40	4.16	12.77	8.34	0.0004	2.07 soluble carrier family 2 (facilitated glucose transporter), member 3
BTNGA1	19.40	2.07	9.38	5.92	0.0003	2.07 heparin, antibody 2, member A1
AF026166	23.60	5.80	13.85	9.79	0.0020	2.07 chaperon containing TCP1, subunit 2 (beta)
CLT2	17.00	7.88	8.23	5.93	0.0004	2.07 glutathione S-transferase
GLUL	17.00	2.32	3.42	3.42	0.0002	2.07 phen. dienease associated protein
PNN	10.80	1.48	3.23	3.07	0.0006	2.06 Fas-activated serine/threonine kinase
FASTK	10.80	2.39	3.23	3.07	0.0006	2.06 suppressor of 5, carboxylate per2
HSGT1	10.80	14.87	31.00	22.32	0.0040	2.06 vipes-1 Y transglutinin virus related oncogene homolog
LYN	10.80	4.85	7.15	4.41	0.0002	2.06 zinc finger protein 276
ZNF278	10.80	1.58	4.42	2.41	0.0002	2.06 KIA0370 protein
KIA0370	14.80	2.88	7.08	4.42	0.0013	2.06 KIA0370 protein
AB023368	14.80	2.30	7.08	5.06	0.0006	2.06 KIA0370 protein
W09022	115.20	18.19	55.65	30.05	0.0002	2.06 Cluster lnc W29732; 5067 Human retina cDNA (randomly primed sublibrary Homo sapiens cDNA, mRNA sequence).
NOT56L	13.00	2.24	4.23	2.07	0.0007	2.06 KIA1046 protein
KIA1046	13.00	2.24	4.23	2.07	0.0007	2.06 KIA1046 protein
AB028959	109.80	24.83	53.31	35.41	0.0032	2.06 chromosome 22 open reading frame 4
C22ORF4	109.80	24.83	53.31	35.41	0.0032	2.06 Homo sapiens mRNA: cDNA DKFZ55842.119 (from clone DKFZ55842.119)
AL049411	12.20	1.92	5.92	3.75	0.0004	2.06 LPS-induced TNF-alpha factor
AL120815	32.00	5.24	15.54	11.48	0.0008	2.06 exportin, RNA (nuclear export receptor for RNAs)
PIG7	18.20	2.28	8.85	4.08	0.0000	2.06 exportin, RNA (nuclear export receptor for RNAs)
XPO7	18.20	2.28	8.85	4.08	0.0000	2.06 exportin, RNA (nuclear export receptor for RNAs)
AB023195	69.80	14.76	33.85	18.59	0.0020	2.06 KIA0370 protein
AB023195	69.80	14.76	33.85	18.59	0.0020	2.06 KIA0370 protein
AL022398	112.40	25.21	54.89	31.85	0.0028	2.06 Cluster and AL022398; Homo sapiens DNA sequence from PAC 43A014 on chromosome 1q32.3-41. Contains the HSD11B1 gene for Hydroxysteroid (11-beta
37137	2997.16	8.88	20.15	13.50	0.0006	2.05 septin 3
CDBA	15.00	3.87	7.31	4.82	0.0049	2.05 CD8 antigen, alpha polypeptide (p32)
KIA0128	15.00	3.87	7.31	4.82	0.0049	2.05 CD8 antigen, alpha polypeptide (p32)
D59918	33.00	5.70	16.08	12.03	0.0011	2.05 KIA0128 protein; septin 2
LOC84115	15.00	4.08	7.31	4.57	0.0091	2.05 up-regulated by BCG-CWS
AL049893	15.00	4.08	7.31	4.57	0.0091	2.05 up-regulated by BCG-CWS
NFYA	64.00	15.22	26.31	18.11	0.0099	2.05 nuclear transcription factor Y, alpha
AL031778	64.00	15.22	26.31	18.11	0.0099	2.05 nuclear transcription factor Y, alpha
FL20552	20.20	5.02	9.85	8.00	0.0067	2.05 hypophyseal protein FL20552
AB07877	20.20	5.02	9.85	8.00	0.0067	2.05 hypophyseal protein FL20552
AL068111	14.20	3.70	6.92	3.20	0.0071	2.05 Homo sapiens mRNA: cDNA DKFZ55862222 (from clone DKFZ55862222)
CCN1	11.20	2.86	5.46	3.78	0.0073	2.05 carboxyl caseinase malformations 1
IFNGR2	43.20	8.95	21.08	14.59	0.0021	2.05 interferon gamma receptor 2 (interferon gamma transducer 1)
U09475	21.60	3.21	10.54	7.14	0.0004	2.05 SC-INTERACTING PROTEIN
AF045184	21.60	3.21	10.54	7.14	0.0004	2.05 SC-INTERACTING PROTEIN
CHRNB1	10.40	2.61	5.08	3.23	0.0035	2.05 cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)
U09759	10.40	2.61	5.08	3.23	0.0035	2.05 cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)
MAPK9	57.80	8.56	28.15	17.47	0.0003	2.05 mitogen-activated protein kinase 9
DA1	57.80	8.56	28.15	17.47	0.0003	2.05 diaphorase (NADH) (cytochrome b-5 reductase)
ZNF1A1	148.40	8.20	72.54	50.90	0.0001	2.05 diaphorase (NADH) (cytochrome b-5 reductase)
U04062	50.20	9.63	24.54	17.46	0.0015	2.05 zinc finger protein, subfamily 1A, 1 (neuro)
M84382	50.20	9.63	24.54	17.46	0.0015	2.05 zinc finger protein, subfamily 1A, 1 (neuro)
AF081195	30.20	8.04	14.77	7.79	0.0007	2.05 lamin B2
RASGRP1	30.20	8.04	14.77	7.79	0.0007	2.05 RAS guanyl releasing protein 1 (calcium and DAG-regulated)
AF081195	30.20	8.04	14.77	7.79	0.0007	2.05 RAS guanyl releasing protein 1 (calcium and DAG-regulated)
CNP	18.40	2.97	9.00	4.74	0.0003	2.04 2'-3'-cyclic nucleotide 3' phosphodiesterase
KIA0070	18.40	2.97	9.00	4.74	0.0003	2.04 2'-3'-cyclic nucleotide 3' phosphodiesterase
AB020677	31.60	4.16	15.46	10.92	0.0003	2.04 KIA0070 protein
Y10798	31.60	4.16	15.46	10.92	0.0003	2.04 KIA0070 protein
Y10319	41.80	7.48	20.46	12.17	0.0007	2.04 melanoma-associated antigen recognised by cytotoxic T lymphocytes
D87442	33.20	3.58	6.48	4.29	0.0083	2.04 carboxylcaseinase translocase
AB095302	33.20	3.58	6.48	4.29	0.0083	2.04 carboxylcaseinase translocase
AF047448	11.00	2.45	5.38	3.25	0.0078	2.04 luteinase dehydrogenase complex, subunit D, integral membrane protein
K30151	11.00	2.45	5.38	3.25	0.0078	2.04 luteinase dehydrogenase complex, subunit D, integral membrane protein
AF047448	11.00	2.45	5.38	3.25	0.0078	2.04 luteinase dehydrogenase complex, subunit D, integral membrane protein
K30151	11.00	2.45	5.38	3.25	0.0078	2.04 luteinase dehydrogenase complex, subunit D, integral membrane protein
AF047448	11.00	2.45	5.38	3.25	0.0078	2.04 luteinase dehydrogenase complex, subunit D, integral membrane protein
K30151	11.00	2.45	5.38	3.25	0.0078	2.04 luteinase dehydrogenase complex, subunit D, integral membrane protein
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AF047448	11.00	2.45	5.38	3.25	0.0078	2.04 luteinase dehydrogenase complex, subunit D, integral membrane protein
K30151	11.00	2.45	5.38	3.25	0.0078	2.04 luteinase dehydrogenase complex, subunit D, integral membrane protein
AF047448	11.00	2.45	5.38	3.25	0.0078	2.04 luteinase dehydrogenase complex, subunit D, integral membrane protein
K30151	11.00	2.45	5.38	3.25	0.00	

Table 7

PPSP1	D08850	11.40	2.88	5.82	3.75	0.0062	2.03 phosphatidyl inositol-3-kinase synthase 1
IL11RA	U32324	24.20	3.83	11.92	6.42	0.0003	2.03 Interleukin 11 receptor, alpha
KIA0276	D87468	12.80	1.48	8.31	3.88	0.0001	2.03 KIA0276 protein
HNRPD	U94630	49.00	8.37	24.15	14.56	0.0008	2.03 heterogeneous nuclear ribonucleoprotein D (A-U-rich element RNA-binding protein 1, 37kD)
RAB1	AL050268	19.80	3.07	9.77	7.49	0.0019	2.03 RAB1, member RAS oncogene family
DJ1043K10.2	AL022228	10.60	2.07	5.23	2.88	0.0013	2.03 hypothetical protein
DOOST	D28843	24.00	6.24	11.85	8.49	0.0078	2.03 dactyl-4-phosphopentacetic-acid-protein glycosyltransferase
ERN1	D28843	24.00	6.20	11.85	7.34	0.0068	2.03 enhancer of rudimentary (Drosophila) homolog
RPS8K43	U03016	187.20	31.80	82.54	48.48	0.0011	2.03 ribosomal protein S8 kinase, 60kD, polypeptide 3
DNFZP56016	AL039458	13.40	3.38	6.82	5.47	0.0079	2.03 DNFZP5601624 protein
NTE	A004632	40.80	4.44	20.15	10.48	0.0006	2.02 neurotrophin target esterase
MGSA5	AB011579	29.00	5.10	12.85	7.17	0.0013	2.02 meningioma expressed antigen 5 (hyaluronidase)
UNK_A117678	AA178780	61.80	15.93	30.45	16.03	0.0089	2.02 Cluster ind AY176780: ap32a10.61 Stragogene neurospineinellum (p937231) Homo sapiens cDNA clone IMAGE51130 3' similar to contains Alu repetitive elem
RERE	AB007927	14.00	3.24	6.92	4.82	0.0042	2.02 arginine-glycine acid dipeptide (REG) repeats
KAA11115	AB028938	37.00	6.96	18.31	12.81	0.0018	2.02 KAA11115 protein
IF16	D63878	183.20	40.83	80.77	56.67	0.0083	2.02 KIA0154 protein; ADP-ribosylation factor binding protein GGA3
RNF15	U90547	16.00	3.32	7.92	4.88	0.0020	2.02 interferon, gamma-inducible protein 16
MOF	AL050395	16.00	2.45	7.92	5.74	0.0007	2.02 ring finger protein 15
DSIF1	Z50781	43.80	11.17	21.69	14.88	0.0067	2.02 member of MYST family histone acetyl transferases, homolog of Drosophila MOF
KAA0981	AB023198	55.60	13.59	27.54	20.04	0.0059	2.02 KIA0981 protein
FLJ20297	AF052134	19.40	4.28	9.62	5.26	0.0078	2.02 hypothetical protein FLJ20297
ADAM10	Z48579	15.20	3.27	7.54	5.90	0.0038	2.02 a disintegrin and metalloproteinase domain 10
KAA0543	AB011115	30.40	7.86	15.08	9.54	0.0071	2.02 KIA0543 protein
VEGF8	U43398	22.80	2.95	11.31	7.30	0.0002	2.02 vascular endothelial growth factor 8
KAA0747	AB018290	56.60	14.79	28.08	23.28	0.0097	2.02 KAA0747 protein
UNK_AL00759	AL047596	41.40	7.64	20.54	11.84	0.0009	2.02 Homo sapiens cDNA: FLJ2480 f8, clone COL05204
UNK_AL050378	AL050378	33.80	5.88	16.77	11.40	0.0010	2.02 Homo sapiens mRNA: cDNA DKFZ556J101 (from clone DKFZ556J101)
BIN1	AF001383	29.80	7.08	14.69	9.86	0.0050	2.01 biding integrator 1
CSF66	AF020043	23.40	5.59	11.82	8.28	0.0052	2.01 chondrin sulfate proteoglycan 6 (barnase)
H26	U08831	17.20	3.70	8.54	5.25	0.0028	2.01 H26
UP	X60836	34.40	7.63	17.00	12.94	0.0044	2.01 uridine phosphorylase
TPST2	AF049891	111.20	13.41	55.23	32.14	0.0001	2.01 lysosomal sulfamidase 2
CLZT751	AF070530	17.80	2.17	6.05	4.82	0.0001	2.01 lysosomal protein, clone 24751
PRBP1	L77884	13.00	3.16	6.46	4.41	0.0055	2.01 amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe5)
KRSPR	AA028946	13.00	2.12	6.46	4.88	0.0012	2.01 KH-type splicing regulatory protein (FUSE binding protein 2)
COP3	AF031647	21.20	3.96	10.54	7.09	0.0014	2.01 COP3 (constitutive photomorphogenic, Arabidopsis, homolog) subunit 3
ROCK1	U43195	24.80	3.85	12.23	7.79	0.0005	2.01 Rho-associated, coiled-coil containing protein kinase 1
BAP1	AF043561	16.40	4.28	8.15	4.43	0.0074	2.01 BRCA1 associated protein-1 (ubiquitin carboxyl-terminal hydrolase)
CRBP	D78134	16.40	4.16	8.15	5.85	0.0069	2.01 cold inducible RNA-binding protein
DNFZP56002	AL096741	23.20	5.93	11.54	8.54	0.0077	2.01 hypothetical protein
PAPSS1	Y10287	40.20	9.20	20.00	10.89	0.0036	2.01 3-prime-phosphoadenosine 5-prime-phosphosulfate synthase 1
MAF	AF055376	68.60	14.62	33.15	23.10	0.0034	2.01 v-maf myeloblastic/neurotic fibrosarcoma (avian) oncogene homolog
HLA-DQB1	M18276	48.20	8.53	24.00	20.43	0.0028	2.01 major histocompatibility complex, class II, DQ beta 1
ITPR3	U01082	12.20	1.48	6.08	3.25	0.0001	2.01 Insitol 1,4,5-trisphosphate receptor, type 3
DTRK1A	D85550	58.20	13.33	29.00	17.54	0.0037	2.01 dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A
C22	J04132	31.80	7.82	15.85	10.90	0.0038	2.01 CD32 antigen, zeta polypeptide (IT13 complex)
AES	A051946	23.00	3.48	11.46	4.99	0.0002	2.01 amino-terminal enhancer of split
PTPN1	K31724	28.40	6.35	14.15	9.88	0.0037	2.01 protein tyrosine phosphatase, non-receptor type 1
AD01	H21154	19.80	4.04	9.77	5.69	0.0020	2.01 S-adenosylmethionine decarboxylase 1
KAA0810	AB018353	68.20	12.56	33.00	19.00	0.0012	2.01 KIA0810 protein
KAA0107	D14663	25.80	6.47	12.77	9.02	0.0070	2.00 KIA0107 gene product
PRSS15	XT8040	155.80	18.32	77.62	48.68	0.0001	2.00 protease, serine, 15
NR3C1	M10901	200.60	28.11	100.08	61.50	0.0003	2.00 nuclear receptor subfamily 3, group C, member 1
RNF6	AL010246	33.60	5.68	16.77	10.05	0.0006	2.00 ring finger protein (C3HC3 type) 6
ISG20	U88964	28.20	7.46	14.08	10.44	0.0092	2.00 interferon stimulated gene (ISG20)
CFB	U22852	15.40	2.81	7.69	4.75	0.0007	2.00 Cluster ind U22852: Human T-lymphocyte specific protein tyrosine kinase p56lck (lck) aberrant mRNA, complete cds.
ATPSJ	AA045575	17.40	4.34	8.69	7.30	0.0087	2.00 core-binding factor, beta subunit
UNK_AF02326	AF023268	33.40	6.23	16.69	12.11	0.0018	2.00 ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6
UNK_J060311	U09311	35.40	9.10	17.69	11.65	0.0073	2.00 Homo sapiens cDNA: FLJ23260 f8, clone COL05804, highly similar to HSJ09011 Human clone 23632 mRNA sequence
BUC36	AB057607	16.00	4.08	8.00	5.70	0.0074	2.00 uncharacterised bone marrow protein BM036
JMJ1	AL021838	16.00	3.67	8.00	5.23	0.0041	2.00 jumonji (mouse) homolog
USP9X	X08288	18.00	2.12	8.00	9.06	0.0045	2.00 ubiquitin specific protease 9, X chromosome (Drosophila fa) (adult related)

Table 8

Gene Name	Accession #	p value	ave normality	sd normality	ave chronic	sd chronic	avg le chonulin	gene description
CCT3	X74801	0.009937	2.75	1.83	35.20	27.31	12.80	CCT3
GRHR	U74804	0.009949	2.38	1.06	18.40	15.22	7.75	glucocorticoid-induced leucine aminopeptidase
ZNF212	U38864	0.008821	3.38	0.92	12.80	6.31	3.73	zinc finger protein 212
RPS3	X55715	0.010192	2.88	2.64	10.80	1.67	3.88	RPS3
SPARC	J03040	0.009001	4.13	4.32	10.00	2.55	2.42	secreted protein, acidic, cysteine-rich (osteonectin)
HLA-DPB1	M33684	0.009253	6.25	3.92	14.20	5.81	2.37	major histocompatibility complex class II, DP beta 1
RAN	M31469	0.009278	8.50	5.15	21.40	7.89	2.25	RAN, member RAS oncogene family
GSN	X04412	0.00832	6.13	2.59	13.60	5.37	2.22	GSN
KIA0092	AB023209	0.009415	4.63	1.51	10.20	6.59	2.21	KIA0092 protein
SEC14L1	D87029	0.009467	12.88	5.72	28.20	10.99	2.19	SEC14 (S. cerevisiae)-like 1
PLTP	L28232	0.009516	6.88	4.36	15.00	5.86	2.18	phospholipid transfer protein
VIM	Z19554	0.009543	19.50	8.50	41.20	9.83	2.11	VIM
PDRR53	AF060228	0.009658	22.75	9.27	47.20	19.46	2.07	retinoic acid receptor responder (larazotol induced) 3
UNK_L27065	L27065	0.009648	6.00	5.07	12.40	6.58	2.07	Neurofibromin 2 Tumor Suppressor
SAH	X00662	0.003295	18.00	8.49	9.00	1.22	2.07	SAH
MAH5	AF010607	0.001577	28.00	15.10	14.00	2.00	0.50	MAD (mothers against decapentaplegic, Drosophila) homolog 5
IGHMBP2	L14754	0.004237	19.63	9.13	9.80	2.17	0.50	Immunoglobulin mu binding protein 2
XBP1	Z59350	0.004362	25.25	13.09	12.60	4.04	0.50	X-box binding protein 1
CALCR	L00587	0.004237	51.75	25.04	25.80	8.58	0.50	calcitonin receptor
MDF1	U78313	0.003068	62.63	30.50	31.00	9.82	0.50	calcitonin receptor
UNK_AL050030	AL050030	0.003109	13.38	5.18	6.50	2.19	0.49	UNK_AL050030
UNK_W25326	W25326	0.001916	53.88	24.79	26.20	11.03	0.49	Homo sapiens DNA, cosmid clones TNE2 and TNE2
TUBA3	X01703	0.001219	12.50	5.63	6.00	1.22	0.48	TUBA3
SPUF	A4883101	0.007552	65.25	33.80	30.40	7.86	0.47	secreted protein of unknown function
NFE2L1	L24123	0.006548	28.38	13.78	13.20	2.05	0.47	NFE2L1
COL19A1	AF018081	0.004472	61.38	32.49	28.40	4.51	0.46	collagen, type XVIII, alpha 1
W27871	W27871	0.004279	42.63	20.56	18.60	2.70	0.46	Cluster lnd W27871: 33a1 Human retina cDNA, randomly primed sublibrary Homo sapiens cDNA, mRNA sequence
HSP32	L26336	0.004681	23.63	10.74	10.60	2.68	0.45	heat shock 70kD protein 2
FRAP1	V020031	0.00772	28.86	13.46	12.00	4.90	0.45	ESTs, Weakly similar to atrophin-1 related protein (H.sapiens)
AKR4040	AF080400	0.001603	28.30	16.35	11.80	3.27	0.45	ESTs, Moderately similar to JC-109 pig-c protein - human (H.sapiens)
AKR4047	AB007946	0.006666	88.50	47.91	39.20	14.41	0.44	KIAA0477 gene product
MEG8	U78062	0.005343	13.13	5.77	5.90	1.79	0.44	RNA polymerase II transcriptional regulation mediator (MeuB, S. cerevisiae, homolog of)
SMN1	A080563	0.005403	24.00	14.08	10.80	3.36	0.44	inostol polyphosphate 5-phosphatase
MLL14	AB011399	0.004237	150.38	70.56	65.80	15.83	0.44	myeloid/lymphoid or mixed-lineage leukemia (t(8;21)(q24;q32))
LLGL1	AW007029	0.004429	12.38	6.93	5.40	1.14	0.44	lethal giant larvae (Drosophila) homolog 1
UNK_W28012	W28012	0.004233	55.00	24.12	24.00	1.00	0.44	Cluster lnd W28012: 55a8 Human retina cDNA, randomly primed sublibrary Homo sapiens cDNA, mRNA sequence
PIGR	X73079	0.003749	87.75	38.30	38.20	8.44	0.44	PIGR
TBL1	AA149637	0.005201	57.25	27.37	24.80	6.22	0.43	transducin beta-like 1
KIAA0710	D85965	0.004846	38.88	18.86	16.80	2.77	0.43	KIAA0710 gene product
LTK	D18105	0.004528	19.00	9.53	8.20	2.66	0.43	leukocyte tyrosine kinase
RPS17	M13932	0.004379	14.38	7.39	6.20	1.49	0.43	ribosomal protein S17
GRSF1	U07231	0.004352	28.88	13.57	12.40	2.07	0.43	G-rich RNA sequence binding factor 1
UNK_AL022388	AL022388	0.004054	20.50	8.70	8.80	3.58	0.43	Cluster lnd AL022388: Homo sapiens DNA sequence from PAC 434D14 on chromosome 1q32.3-41.
GATA2	M77810	0.00394	17.75	10.46	7.60	2.51	0.43	GATA-binding protein 2
PABPC1	Y02045	0.003317	27.63	11.80	11.80	2.77	0.43	PABPC1
MPNOSPH9	N23137	0.002913	10.38	4.83	4.40	1.14	0.42	My-phase phosphoprotein 9
NFAB1	A65603	0.002132	10.38	5.28	4.60	1.14	0.42	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
SRPG	AB03447	0.00347	59.00	31.72	24.80	7.26	0.42	small nuclear ribonucleoprotein polypeptide G
KRT4A38	X06534	0.001769	11.50	6.07	4.80	1.48	0.42	KRT4A38
CLL1	D06813	0.00245	21.83	10.87	9.00	2.45	0.42	clusterin-like 1 (retinol)
PM52	U13696	0.001807	46.53	20.87	19.40	3.91	0.42	postmeiotic segregation increased (S. cerevisiae) 2
MGAT2	U15128	0.002266	12.13	6.42	5.00	1.22	0.41	mannosyl (alpha-1,6)-polyacornish beta-1,2-N-acetylglucosaminyltransferase
UBE2L6	A4881502	0.003066	15.63	7.82	6.40	1.14	0.41	ubiquitin-conjugating enzyme E2L 6
GYPE	X53004	0.002639	16.13	8.67	6.60	2.07	0.41	GYPE
IRS1	S62539	0.001656	89.38	43.55	36.40	12.56	0.41	insulin receptor substrate 1
ITGB3	M25108	0.001833	10.50	6.78	4.20	0.84	0.40	integrin, beta 3 (platelet glycoprotein IIb, antigen CD61)
CDK5R1	X00043	0.004472	16.63	7.93	6.20	3.19	0.37	CDK5R1
UNK_A157322	A157322	0.004237	17.25	8.58	6.40	2.07	0.37	Cluster lnd A157322: P72.1, 16_111/turno2 Homo sapiens cDNA 3', mRNA sequence.
LOC55977	AF070843	0.004237	32.38	17.14	12.00	1.73	0.37	LOC55977
HPK3	AF044849	0.002843	11.38	6.48	4.20	1.30	0.37	homodimer-interacting protein kinase 3
PTPN21	X78510	0.002454	17.88	10.92	6.60	1.14	0.37	PTPN21

Table 8

UNK_X89059	5.03E-05	21.13	10.70	7.40	1.14	0.35	STK9
KIA0744	0.00017	18.88	11.47	6.60	2.51	0.35	KIA0744 gene product; histone deacetylase 7
SLC18A2	0.001401	23.88	18.39	8.20	1.92	0.34	solute carrier family 18 (vesicular monoamine), member 2
CELSR1	0.000278	25.25	13.22	8.40	2.19	0.33	cadherin EGF LAG seven-pass G-type receptor
L33410	0.001154	48.25	23.27	15.00	3.00	0.32	thrombospondin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development) factor
AB002386	0.000908	10.50	6.14	3.40	0.55	0.32	KIA0368 protein
W25917	0.001177	16.75	10.05	5.40	1.67	0.32	Cluster 1 and W25917: 14kb Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence
CKADR	0.000704	10.63	8.86	3.40	1.14	0.32	CKADR
X58965	0.000814	54.00	28.17	18.40	2.88	0.30	NME2
AB018194	0.000592	18.50	9.19	5.60	1.67	0.30	ELK1, member of ETS oncogene family
AF054187	0.000396	11.25	7.34	3.40	1.14	0.30	nascim-poly(amide)-associated complex alpha polypeptide
U09413	0.000457	21.25	11.36	6.40	1.52	0.30	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3
U11700	0.000559	14.75	8.38	4.40	0.89	0.30	ATPase, Cu++ transporting, beta polypeptide (Wilson disease)
AJ523538	0.000553	23.50	12.71	7.00	2.45	0.30	homodomain-hitchhiking protein kinase 3
U96629	0.000317	12.13	6.56	3.60	0.89	0.30	UNK_U96629
D85378	6.29E-05	12.88	6.94	3.80	1.30	0.30	thyrotrophin-releasing hormone receptor
U25441	9.9E-06	30.50	15.69	9.00	3.67	0.30	dopamine receptor D3
U42391	1.44E-05	10.88	7.28	3.20	1.64	0.29	myosin IXB
AL049470	2.28E-05	12.25	6.69	3.60	1.67	0.29	HYPB
X88176	0.000349	11.63	6.25	3.40	1.14	0.29	CASP8
AJ010232	6.98E-05	43.13	23.24	12.80	5.13	0.29	PFPL3
L10338	2.89E-05	18.63	9.53	4.80	2.39	0.29	sodium channel, voltage-gated, type I, beta polypeptide
D87468	3.48E-05	24.25	17.43	7.00	3.32	0.29	activity-regulated cytoskeleton-associated protein
X52541	4.09E-05	10.50	6.52	3.00	0.00	0.29	early growth response 1
M21259	0.000202	16.25	8.83	4.60	1.52	0.28	small nuclear ribonucleoprotein polypeptide E
U59111	9.61E-05	17.00	9.50	4.80	0.84	0.28	dermatan sulphate proteoglycan 3
L10717	0.000154	10.13	7.95	2.80	1.30	0.28	IL2-inducible T-cell kinase
M88740	4.18E-05	10.13	5.69	2.80	1.30	0.28	NHLH2
D68864	9.93E-05	17.38	9.26	4.80	1.48	0.28	acyl-LDL receptor, SREC
AJ236865	0.000339	10.88	9.72	3.00	0.71	0.28	ZNF148
U66817	0.000116	13.88	7.77	3.80	1.30	0.27	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1
U50393	0.000194	10.63	6.44	2.60	0.55	0.24	retinoic acid responsive
M20842	0.000122	14.00	6.41	3.40	1.52	0.24	Human alkali myosin light chain 3 mRNA, complete cds
S40719	0.000161	10.75	6.94	2.80	0.89	0.24	glial fibrillary acidic protein
AB020654	0.000141	14.38	8.02	3.40	0.89	0.24	KIA0847 protein

Table 9

Genes Differentially regulated in Murine EAE and Human MS		
Gene Name	Murine Accession	Description
EEF1D	aa253918	Cluster Incl Z21507: H.sapiens EF-1delta gene encoding human elongation factor-1-delta.
PIM2	Msa.2067.0	Human pim-2 protooncogene homolog pim-2h mRNA, complete cds.
PRDX2	Z21848	Cluster Incl L19185: Human natural killer cell enhancing factor (NKEFB) mRNA, complete cds.
SEC24C	aa175517	Cluster Incl D38555: Human mRNA for KIAA0079 gene, complete cds.
UNK_AJ2454	u85207	Cluster Incl AJ245416: Homo sapiens mRNA for G7b protein (G7b gene, located in the class III region of the major histocompatibility complex.
XIP	Msa.3679.0	Cluster Incl AF029890: Homo sapiens hepatitis B virus X interacting protein (XIP) mRNA, complete cds.

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Table 10

	A	B	C	D	E
1	Peripheral Blood Genes Predictive of Multiple Sclerosis				
2					
3	Gene	Observed	Random Perm 1%	Random Perm 5%	Random Perm (Median)
4	FOS	2.562527	1.4817816	1.1614933	0.7007793
5	U19261	2.114882	1.3043897	0.87986296	0.53156734
6	DUSP1	1.939225	1.2410891	0.8299547	0.5107011
7	KIAA0906	1.87203	1.2133218	0.80576986	0.48326102
8	MAP4K1	1.860437	1.2085855	0.79408497	0.47091565
9	DGKZ	1.837408	1.2069645	0.77625513	0.4690797
10	SNRP70	1.81934	1.1756094	0.7738778	0.45766842
11	ETR101	1.801398	1.1731951	0.76643366	0.440602
12	KIAA0864	1.775063	1.1709846	0.7597211	0.43995833
13	SFRS8	1.77253	1.1582581	0.7580478	0.43687758
14	AI890903	1.764722	1.1560028	0.7550534	0.4319513
15	JUNB	1.752881	1.1488212	0.74930257	0.4269654
16	STAT4	1.719926	1.1444192	0.7461597	0.41349095
17	AQP3	1.705897	1.130565	0.7446831	0.4113997
18	HSU79253	1.664187	1.1186187	0.7290762	0.4062087
19	BIN1	1.649725	1.1111517	0.7287055	0.402228
20	ATM	1.641806	1.1030456	0.7274922	0.39897352
21	EDG4	1.634613	1.0987875	0.7267664	0.39316127
22					
23	Prediction based on correlation metrics proposed by Gollub et. al. (1999)				

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WHAT IS CLAIMED:

1. A method of diagnosing a subject with multiple sclerosis, the method comprising the step of comparing:
 - a) a level of expression of a marker in a sample from the subject, wherein the marker is selected from the group consisting of markers listed in Tables 7-10, and
 - b) a normal level of expression of the marker in a control sample,
wherein a substantial difference between the level of expression of the marker in the sample from the subject and the normal level is an indication that the subject is afflicted with multiple sclerosis.
2. The method of claim 1, wherein the marker corresponds to a transcribed polynucleotide or a portion thereof.
3. The method of claim 2, wherein the sample is collected from brain tissue.
4. The method of claim 2, wherein the sample is peripheral blood mononuclear cells.
5. The method of claim 1, wherein the control sample is from a non-diseased subject and the substantial difference is a factor of at least about 2.
6. The method of claim 1, wherein the control sample is from non-involved tissue of the subject and the substantial difference is a factor of at least about 2.

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7. The method of claim 1, wherein the control sample is from non-involved tissue of the subject and the substantial difference is a factor of at least about 5.

8. The method of claim 1, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a protein corresponding to the marker.

9. The method of claim 8, wherein the presence of the protein is detected using a reagent which specifically binds with the protein.

10. The method of claim 9, wherein the reagent comprises an antibody or fragments thereof.

11. The method of claim 1, wherein the marker is selected from the markers listed in Table 9.

12. The method of claim 1, wherein the marker is selected from the markers listed in Table 10.

13. The method of claim 1, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or portion thereof, wherein the transcribed polynucleotide comprises the marker.

14. The method of claim 13, wherein the transcribed polynucleotide is a mRNA.

15. The method of claim 13, wherein the transcribed polynucleotide is a cDNA.

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16. The method of claim 1, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or a portion thereof which hybridizes with a labeled probe under stringent conditions, wherein the transcribed polynucleotide comprises the marker.

17. A method of diagnosing a subject with multiple sclerosis, the method comprising the step of comparing:

- a) a level of expression in a sample of the subject of each of a panel of markers independently selected from the markers listed in Tables 7-10, and
- b) a normal level of expression of the panel of markers obtained from a control sample,

wherein the level of expression of the panel of markers is substantially different, relative to the corresponding normal level of expression of the panel of markers, indicates that the subject is afflicted with multiple sclerosis.

18. The method of claim 17, wherein the panel of markers comprises at least 5 markers.

19. The method of claim 17, wherein the control sample is from a non-diseased subject.

20. The method of claim 17, wherein the control sample is from non-involved tissue of the subject.

21. The method of claim 17, wherein the panel of markers comprises markers listed in Table 10.

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22. A method for monitoring the progression of multiple sclerosis in a subject, the method comprising the steps of:

a) detecting in a subject sample at a first point in time, a level of expression of at least one marker, wherein the at least one marker is selected from the group consisting of markers listed in Tables 7-10;

b) repeating step a) at a subsequent point in time with the at least one marker;

c) detecting a substantial difference between the levels of expression detected in steps a) and b);

wherein the substantial difference between the levels of expression indicates that the subject has progressed to a different stage of multiple sclerosis.

23. The method of claim 22, wherein at least 5 markers are selected from the group of markers listed in Tables 7-10.

24. The method of claim 22, wherein the at least one marker corresponds to a transcribed polynucleotide or portion thereof.

25. The method of claim 24, wherein the samples are collected from brain tissue.

26. The method of claim 24, wherein the samples are peripheral blood mononuclear cells.

27. A method of assessing the efficacy of a test compound for inhibiting multiple sclerosis in a subject, the method comprising the step of comparing:

a) expression of a marker in a first sample obtained from the subject and exposed to the test

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compound, wherein the marker is selected from the group consisting of markers listed in Tables 7-10, and

b) expression of the same marker in a second sample obtained from the subject, wherein the second sample is not exposed to the test compound,

wherein a substantially different level of expression of the marker in the first sample, relative to the second sample, is an indication that the test compound is efficacious for inhibiting multiple sclerosis in the subject.

28. The method of claim 27, wherein the first and second samples are portions of a single sample obtained from the subject.

29. The method of claim 28, wherein the level of expression in the first sample approximates the level of expression in a control sample.

30. A method of assessing the efficacy of a therapy for inhibiting multiple sclerosis in a subject, the method comprising the steps of comparing:

a) expression of a marker in a first sample obtained from the subject prior to providing at least a portion of the therapy to the subject, wherein the marker is selected from the group consisting of markers listed in Tables 7-10, and

b) expression of the marker in a second sample following provision of the portion of the therapy,

wherein a substantially different level of expression of the marker in the second sample, relative to the first sample, is an indication that the test compound is efficacious for inhibiting multiple sclerosis in the subject.

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31. The method of claim 30, wherein a substantially similar level of expression in the second sample, relative to the control sample, is an additional indication that the test compound is efficacious for inhibiting multiple sclerosis.

32. The method of claim 31, further comparing expression of the marker in a control sample, wherein expression of the marker in the second sample is similar to expression of the marker in the control sample.

33. A method of screening test compounds for inhibitors of multiple sclerosis, the method comprising the steps of:

- a) obtaining a sample comprising cells from the subject;
- b) separately maintaining aliquots of the sample in the presence of a plurality of test compounds;
- c) comparing the expression levels of a marker in each of the aliquots, wherein the marker is selected from the group consisting of markers listed in Tables 7-10; and
- d) selecting one of the test compounds which induces a substantially different level of expression of the marker in the aliquot containing that test compound, relative to other test compounds.

34. The method of claim 33, wherein the substantially different level of expression is a substantially lower level of expression.

35. The method of claim 34, wherein the substantially different level of expression is a substantially enhanced level of expression.

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36. A kit for diagnosing a subject with multiple sclerosis, the kit comprising reagents for assessing expression of a marker selected from the group consisting of markers listed in Tables 7-10.

37. A kit for diagnosing multiple sclerosis in a subject, the kit comprising a nucleic acid probe wherein the probe specifically binds with a transcribed polynucleotide corresponding to a marker selected from the group consisting of markers listed in Tables 7-10.

38. A kit for assessing the suitability of each of a plurality of compounds for inhibiting progression of multiple sclerosis in a subject, the kit comprising:

- a) the plurality of compounds; and
- b) a reagent for assessing expression of a marker selected from the group consisting of markers listed in Tables 7-10.

39. A kit for diagnosing multiple sclerosis in a subject, the kit comprising an antibody, wherein the antibody specifically binds with a protein corresponding to a marker selected from the group consisting of markers listed in Tables 7-10.

40. A method of modulating a level of expression of a marker selected from the markers listed in Tables 7-10, the method comprising providing to diseased cells of a subject an antisense oligonucleotide complementary to a polynucleotide corresponding to the marker.

41. A method of modulating a level of expression of a marker selected from the markers listed in Tables 7-10, the method comprising providing to diseased cells of a subject a protein.

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42. The method of claim 41, wherein the protein is provided to the cells by providing a vector comprising a polynucleotide encoding the protein to the cells.

43. A method of modulating a level of expression of a marker selected from the markers listed in Tables 7-10, the method comprising providing to diseased cells of a subject an antibody.

44. The method according to claim 43, wherein the method further comprises a therapeutic moiety conjugated to the antibody.

45. A method of localizing a therapeutic moiety to diseased tissue comprising exposing the tissue to an antibody which is specific to a protein encoded from a marker listed in Tables 7-10.

46. A method of screening for a test compound capable of modulating the activity of a protein encoded from a marker listed in Tables 7-10, the method comprising combining the protein and the test compound, and determining the effect of the test compound on the therapeutic efficacy of the protein.

47. A method of screening for a bioactive agent capable of interfering with the binding of a protein encoded from a marker listed in Tables 7-10 and an antibody which binds to the protein, the method comprising:

a) combining the protein, a bioactive agent and an antibody which binds to the protein; and

b) determining the binding of the protein or fragment thereof and the antibody.

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48. An antibody which specifically binds to a protein encoded from a marker listed in Tables 7-10.

49. The antibody of claim 48, wherein the antibody is a monoclonal antibody.

50. The antibody of claim 49, wherein the antibody is a humanized antibody.

51. A peptide encoded from markers listed in Tables 7-10.

52. A composition comprising the peptide of claim 51.

53. A composition capable of modulating an immune response in a subject, the composition comprising a protein encoded from a marker listed in Tables 7-10, and a pharmaceutically acceptable carrier.

54. A biochip comprising a panel of markers selected from the group of markers listed in Tables 7-10.

55. The biochip of claim 54, wherein the markers are selected for subjects suspected of having secondary progressive multiple sclerosis.

56. The biochip of claim 54, wherein the markers are selected for subjects suspected of having primary progressive multiple sclerosis.

57. The biochip of claim 54, wherein the markers are selected for subjects suspected of having relapsing-remitting multiple sclerosis.

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58. The biochip of claim 54, wherein the markers are selected for subjects suspected of having multiple sclerosis, wherein the subjects are from a high-risk geographic region.

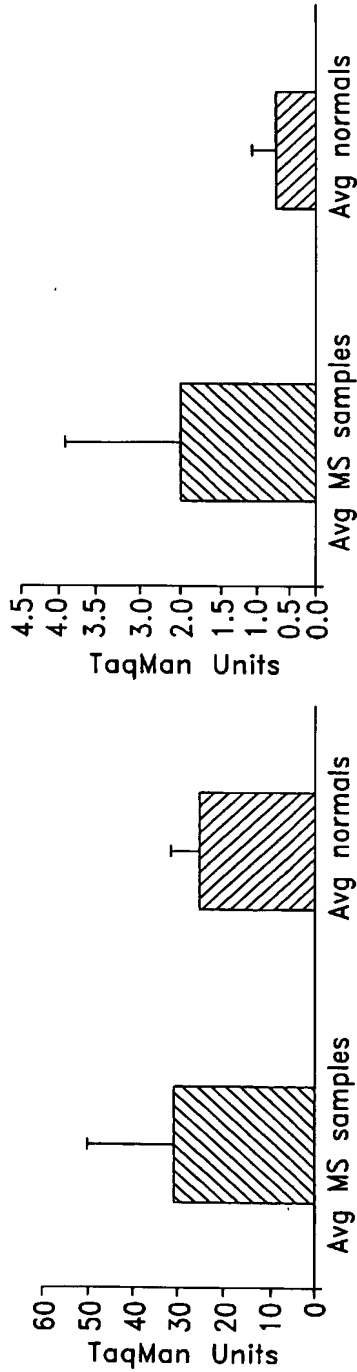


FIG. 1A

FIG. 1B

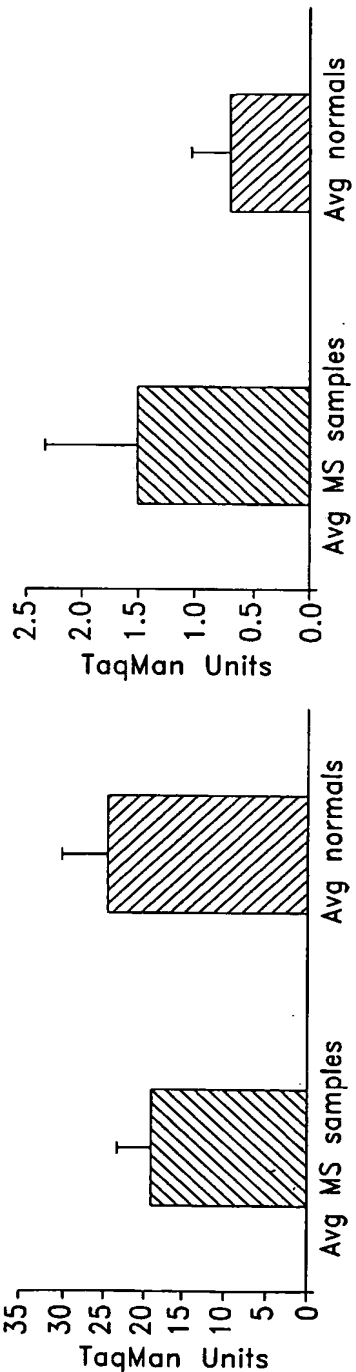


FIG. 1C

FIG. 1D

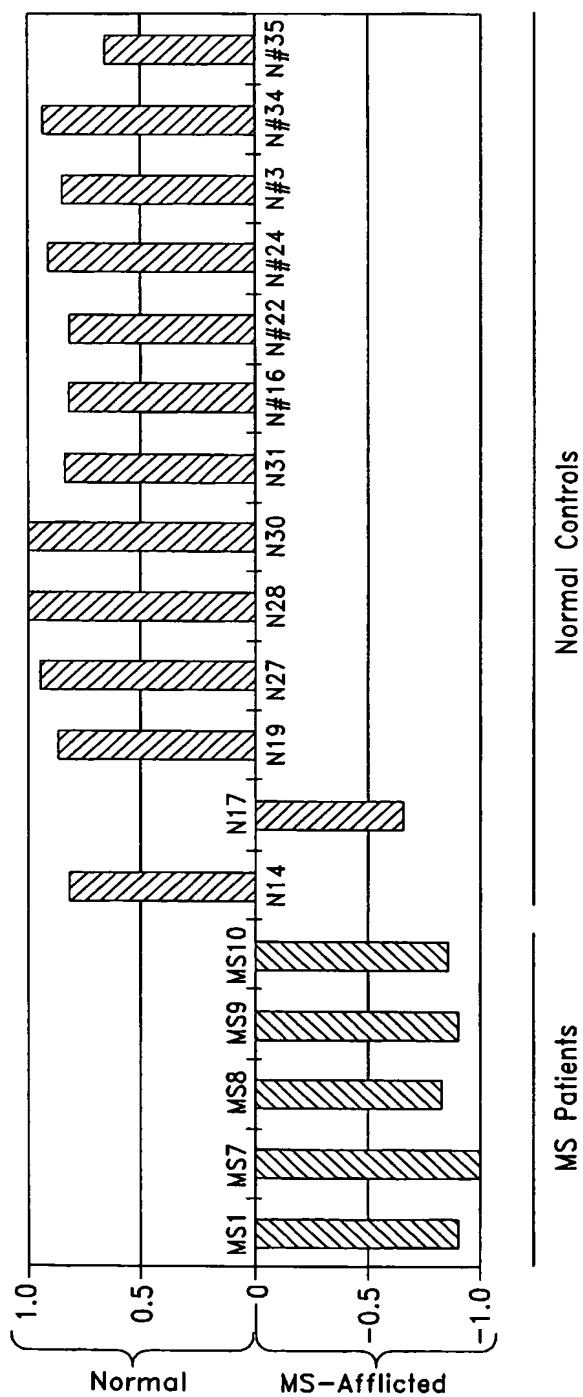


FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/09305

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07H 21/04; C12Q 1/68

US CL : 435/6; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VAN BOXEL-DEZAIRE et al. "Decreased interleukin-10 and increased interleukin-12p40 mRNA are associated with disease activity and characterize different disease stages in multiple sclerosis" Annals of Neurology, June 1999, Vol. 45, No. 6, pages 695-703, see entire document.	1-26
A	SEDLACEK et al. "Evolutionary conservation and genomic organization of XAP-4, an Xq28 located gene coding for a human rab GDP-dissociation inhibitor (GDI)". Mammalian Genome. October 1994, Vol. 5, No. 10, pages 633-639, see entire document.	1-26



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T*
"A" document defining the general state of the art which is not considered to be of particular relevance	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

23 July 2002 (23.07.2002)

Date of mailing of the international search report

22 AUG 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer

Felicia D. Roberts for
Jeanine Encwold Goldberg

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/09305

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-26, in part, namely gene XAP4

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/09305

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group 1-808, claim(s) 1-26, in part, drawn to methods of diagnosing a subject with multiple sclerosis by comparing expression of a marker listed in Tables 7-10 to normal expression levels or using a panel of markers for expression analysis of multiple sclerosis. It is noted that Groups 1-808 correspond to the 808 markers of Tables 7-10. Therefore, the first mentioned invention is the methods of claim 1 to the extent that they apply to expression analysis of XAP4 (Accession U67322) and panels of at least five markers wherein the panel comprises XAP4. Group 1, the first mentioned invention, is the invention which will be searched in accordance with PCT Article 17(3)(a). Additional groups may be elected. For example, if Group 2 is elected, and the proper fees are paid, then claim 1 will be examined to the extent that they apply to methods of expression analysis with respect to OA48-18 (Accession Number AF069250) or a panel of five markers comprising said marker recited in claim 1. Upon election of an invention to be searched in addition to group 1, please identify the number of the gene to be searched in the method claims.

Group 809-1616, claim(s) 27-35, in part, drawn to methods of assessing the efficacy of therapy or compounds by detecting expression of a marker listed in Tables 7-10.

Group 1617-2424, claim(s) 36-38, 54-58 in part, drawn to kits comprising nucleic acids of Tables 7-10 and biochips.

Group 2425-3232, claim(s) 36, 39, 48-50 in part, drawn to kits comprising antibodies which binds with a protein corresponding to the markers of Tables 7-10.

Group 3233-4040, claim(s) 40, drawn to methods of modulating expression using antisense oligonucleotides.

Group 4041-4848, claim(s) 41-42, drawn to methods of modulating expression using a protein of Tables 7-10.

Group 4849-5656, claim(s) 43-44, drawn to methods of modulating expression using an antibody of Tables 7-10.

Group 5657-6464, claim(s) 45, drawn to methods of localizing a therapeutic moiety.

Group 6465-7272, claim(s) 46-47, drawn to methods of screening for test compounds by monitoring expression of protein of Tables 7-10.

Group 7273-8080, claim(s) 51-53, drawn to peptides encoded from markers listed in Tables 7-10.

The inventions listed as Groups 1-89 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

The claims are drawn to detecting expression of numerous genes, in the alternative, as diagnostic of multiple sclerosis. It is well known in the art that various genes are differentially expressed in multiple sclerosis cells such that one may detect a predisposition of MS based upon overexpression of a gene compared to the normal expression level of the gene. The disclosure specifically states that "A study by Whitney and colleagues utilized genetic expression data from one patient to compare MS tissue to non-MS tissue, revealing that about sixty-two genes were differentially expressed, including the Duffy chemokine receptor, interferon regulatory factor-2 and tumor necrosis factor alpha receptor 2." (page 3, lines 5-15). Moreover, it is noted that the art teaches the association of over expression of many genes in MS (Baranzini, J. Immunology Vol 165, pages 6576-6582, 2000). Therefore, there is no special technical feature which links the claims as defined by PCT Rule 13.2, and therefore lack of unity is present between the groups.

Additionally, methods which are comparing expression of one gene are not the same as method comparing expression of a different gene. The expression level of one gene as indicative of MS does not bear any indication of how a different gene will be expressed in cancer cells. The molecules, namely genes, which are relied upon in each of the methods do not share a common structure.

With respect to the markers, namely the polynucleotides and polypeptides, each of the genes provided in Table 7-10 are identified by Accession Number indicating that the sequence of the genes were known in the art. For example, XAP4, Genbank Accession Number U67322, available in 1999 contains both a nucleic acid and a polypeptide sequence. Therefore, the genes themselves are not contributions over the art as exemplified in the Tables themselves.

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The groups comprising polynucleotides, kits, polypeptides, antibodies are additionally drawn to multiple, distinct products lacking the same or corresponding special technical features. The nucleic acids are composed of nucleotides and function in, e.g., methods of nucleic acid hybridization or amplification. These groups are directed to different combinations of nucleic acids which are different from one another and may be employed in different methods. The polypeptides differ in both structure and function from either the nucleic acids or the transgenic organisms. The polypeptides are composed of amino acids linked by peptide bonds and arranged in a complex combination of alpha helices, beta pleated sheets, hydrophobic and hydrophilic domains. The polypeptides also differ in function, e.g., fusion proteins with an enzymatic functions. The antibodies are composed of amino acids linked by peptide bonds, antibodies are glycosylated and their tertiary structure is unique, where four subunits (2 light chains and 2 heavy chains) associated via disulfide bonds into a Y-shaped symmetric dimer. The antibodies function in immunoassays. As products of different sets of Groups differ from each other in structure, function, and effect, they do not belong to a recognized class of chemical compound, or have both a "common property or activity" and a common structure as would be required to show that the inventions are "of a similar nature".

The methods are similarly not the same because they rely upon different products and have different objectives, reagents and results. Therefore, the methods lack unity.

Continuation of B. FIELDS SEARCHED Item 3:

file medline biosis caplus embase scisearch

xap4, xap 4, xap-4 or u67322, hbv associated factor